DIAGNOSTIC TESTS FOR INFANTS AND CHILDREN

CFTRI-MYSORE
1835
Diagnostic tests..

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DIAGNOSTIC TESTS FOR INFANTS AND CHILDREN

DIAGNOSTIC TESTS
FOR INFANTS AND CHILDREN

Principles • Clinical and Laboratory Procedures • Interpretation

by **H. BEHRENDT, M.D.**

CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE
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1949

INTERSCIENCE PUBLISHERS, INC., NEW YORK
INTERSCIENCE PUBLISHERS LTD., LONDON

1835

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L-9c;4:31
N49

CFTRI-MYSORE
1835
Diagnostic tests.

INTERSCIENCE PUBLISHERS, INC., 215 Fourth Ave., New York 3, N. Y.

For Great Britain and Northern Ireland:
INTERSCIENCE PUBLISHERS Ltd., 2a Southampton Row, London W. C. 1

Printed in the United States of America by Mack Printing Co., Easton, Pennsylvania

To

Edith M. Behrendt

PREFACE

This volume represents an endeavor to set forth the principles, technics, and interpretations of clinical and laboratory tests as used in infants and children. Such information has heretofore been available only in widely scattered form, and some of it has been difficult to find. In compiling this book a wide range of readers has been kept in mind: the practitioner in a hurry for specific data; the clinician seeking detailed information for the investigation of a given problem; and the laboratory worker in search of technical data for immediate practical application. These are diversified needs—and the author has attempted to meet them; yet the main objective has been to bring out as clearly as possible the special considerations and technical data made necessary by the physiologic peculiarities of the growing organism.

The author was first struck by the need for such a book when he left the laboratory of the late G. Embden in 1921 to devote himself to research in pediatrics. Since that time, with the hope of eventually compiling and evaluating the data, the material that constitutes the substance of this book has been gathered. The actual writing was done between 1940 and 1948, when the author was associated with the Department of Pediatrics of the New York Post-Graduate Medical School and Hospital.

During every phase of the book's preparation, the twin problems of inclusion and exclusion were encountered. However ardently one strives for objectivity, the final selection of material is inevitably influenced by subjective motives. It is also inevitable that errors will be found, whatever the care taken in preparation. It will be appreciated if such shortcomings are brought to the author's attention.

Grateful acknowledgment is made to the many colleagues whose advice and criticism have been invaluable. The author is particularly indebted to Dr. Kate L. Kogan, who wrote the chapter on psychologic tests, to Dr. Hans Strauss for his collaboration in the preparation of the section on electroencephalography, to Dr. B. Ratner for reading the chapter on immunology, and to Dr. Alexander S. Wiener, who has been kind enough to read and criticize the section on rhesus antigen. Finally, sincere thanks are due Miss Dorothy Hirsch for the preliminary editing, Mrs. Natalie Friedheim for the final editing of the manuscript, and Miss Betty R. Schtab for her tireless secretarial assistance.

H. Behrendt

1165 Park Avenue
New York, N.Y.

November, 1948

INTRODUCTION

Diagnostic tests have evolved through a process of standardization of methods used in clinical research for which, as Hoagland states, "the resources of practically every field of science have been tapped."

If one tries to classify the tests described in this book, he will discover that all the tests could be called function tests. They are either true function tests (for example, tolerance tests, which reveal the response of organs or systems to measured demands upon their working capacity) or they give only indirect evidence of the functional status, disclosing the sequels of aberrant processes in the tissues (thus, the changes in the constituents of blood and urine may provide clues to the nature and the site of existing dysfunction).

In preparing a practical guide to diagnostic testing in children, the greatest obstacle is the wealth of material. Therefore, however inclusive such a book should be, the practical limit of its final size is of fundamental importance, as are the factors of human competence (and incompetence), and of knowledge and attitudes built up through personal experience. The guiding principle throughout has been to include all physiologic functions that have a fair chance of being tested in children, to omit tests of a morphologic nature, to select from among the methods known those that seem most suitable according to available opinion, and to describe the methods selected.

In accordance with these principles, some methods of recognized usefulness in general laboratory practice have been omitted while others of seemingly less importance are described in detail. For instance, many routine procedures of urine, blood, and stool examination were omitted because they were not pertinent to any of the tests selected. This would apply also to laboratory examinations of spinal fluid. As suggested by the previous remarks regarding omission of morphologic tests, a discussion of morphologic hematology has not been given. On the other hand, current trends in pediatric practice have prompted the inclusion of electrocardiography, electroencephalography, and psychologic testing. Tests of the sensory functions have been added in order to call attention to the usefulness of badly neglected procedures.

As a rule, a physiologic review and "pediatric considerations" introduce the chapters, or, when warranted, they introduce a group of tests. Emphasizing the functional aspects, these preliminary sections bring out the physiologic peculiarities of the child and their practical consequences in applying a particular test to children.

In the sections entitled "Procedure," descriptions of methods are given in complete detail even though part of such information is available in texts on laboratory procedures. In a few instances, only short summaries of methods seemed warranted.

Each test method or group of methods is followed by an interpretive section. Results considered normal in children of various age groups and a discussion of the test's differential diagnostic significance are included in the interpretation. It would be possible for the reader to form a useful compilation of biologic data about the child by combining the sections appearing throughout the book that define the "norm" of chemical constituents, physical measurements, and functional responses.

The relative lengths of the chapters is not meant to be an indication of the relative importance of the subjects they cover. Several sections (mineral metabolism, Rh factor, etc.) are considerably longer than they might have been because they include much theoretic and technical information and because these subjects are difficult to treat in a brief manner. Conversely, important chapters (endocrine function, for example) are relatively brief because it has been possible to omit many technical data and, instead, to refer the reader to instructions given elsewhere in the book.

Wherever possible, original work on a given subject is cited. The literature references are to periodicals and books through 1946; later publications are referred to only occasionally. No references are made to manufacturers of the drugs required, of apparatus, etc.

It is hoped, then, that many physicians dealing with children can turn to this volume with the assurance that they will find such guidance and information as they could previously secure only by studying a multitude of books or by searching for source material.

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CHAPTER I

Digestive Function

The peculiarities of the child's gastrointestinal functions concern motor, secretory, and absorptive properties. Many procedures and methods have been used in the investigation of these functions in children, but only a limited number of them are useful to the clinician as diagnostic tests. Among the available methods for revealing abnormal motility or gross lesions, roentgenography still takes first place. The roentgenogram, recording "living anatomy and living disease" (1), is very enlightening, since most signs and symptoms of gastrointestinal disease in children are sequelae of disturbed motor function.

The state of the secretory and absorptive functions of the gastrointestinal tract can be ascertained by chemical and microscopic methods; they comprise tests for gastric secretion, function tests of liver and pancreas—the two accessory glands of the intestinal system—and tests of intestinal absorption.

MOTILITY TESTS

Abnormal gastric retention can be visualized roentgenographically or may be measured by aspirating the gastric contents at regular intervals following a test meal. Roentgenographic examination is not discussed in this book. But for the sake of completeness normal emptying time for the stomach and normal times for passage of a barium meal through the intestinal tract of infants and children are given in Table 1.

Emptying time of the infant's stomach is longer than that of the adult, being particularly prolonged during the first week of life. It has also been demonstrated that passage of a barium meal through the small intestine is slower in the infant than in the adult.

However, since passage time through the entire intestinal tract is essentially the same in both, one concludes that passage of material through the large bowel is more rapid in the infant than in the adult. As for newborn infants, there are marked peculiarities in the passage of material both through the small intestine and the colon.

TABLE 1
Stomach Emptying Time and Barium Meal Progress through Intestinal Tract*

		Emptying time, hrs. after ingestion	Hours after ingestion required for first part of barium to enter	
Age	Test meal		Cecum	Pelvic colon
1–8 days	Barium	5–8 (2)	3–6 (7a)	5–8 (7a)
Up to 1 year	Breast milk	2–3 (3)	7–8 (7b)	10–14½ (4)
	Cow's milk (formula)	3–4 (4)		
1 year and over	Barium	2 (5)		
Adults	Barium	2 (6a)	2½ (6b)	12–14 (6b)

* Figures in parentheses are reference numbers.

GASTRIC SECRETION TESTS

There are few clinical indications for testing the secretory function of the stomach in children, so that gastric analysis is now infrequently done. The diagnostic import of such tests in acute gastrointestinal diseases during infancy and childhood is insignificant, and only in chronic "gastric indigestion" and chronic nutritional disorders in children over one year of age is this diagnostic aid useful. When, however, the pediatrician does want a gastric analysis done, he is almost exclusively interested in data on gastric acidity, since examination for the activity of gastric enzymes (pepsin, rennin, lipase) is only rarely helpful in diagnosis in children. For this reason the latter is not discussed here.

Tests for gastric acidity include determinations for free hydrochloric acid, total acidity, and *p*H. Acidity tests may be carried out on "fasting contents" (pure gastric juice), on gastric contents obtained after ingestion of a test meal, or on gastric juice drawn after stimulation of the gastric mucosa by parenteral injection of histamine. Since normal gastric acidity varies with age, correct interpretation of a test requires that the results be compared with the normal values for the age of the child in question, and that both values be based on identical test methods.

Aside from some adaptation to pediatric requirements, the methods of gastric stimulation and analysis for gastric acidity are alike for children and adults. The composition of the test meal has a direct bearing on the results of the test. For instance, children less than one year old are often given as a test meal their usual milk formula, which is the most physiologic stimulant for this age group. If the test is performed on a specimen of the gastric contents after such a test meal, the results reflect not only the acidity as it existed in the undiluted gastric juice but also the original acidity of the milk formula used and its buffer value. Obviously, the results of such tests are not as reliable as when pure juice is drawn and analyzed after histamine administration. All data also suggest that results obtained with the Ewald and alcohol meals cannot compare in accuracy and clinical significance with the histamine test.

Determination of hydrogen ion concentration (*p*H) adds little to the information on secretory activity, and this test will therefore be discussed only briefly.

Methods

Analysis of the stomach contents is carried out on samples which are obtained after stimulation of the gastric glands by test meals or injection of histamine. The procedure of obtaining samples depends upon the method of stimulation.

Ewald Test. The test described is that of Dietrich and Shelby (8). The test is carried out in the morning, on a fasting stomach. An Ewald tube is introduced through the mouth, size of tube depending on the child's age (for infants, tube No. 22). For children under 2 years a soft nelaton catheter (e.g., French No. 4) is just as good, or even better. After passage of the tube, suction is applied by means of a 50 cc. syringe fitted to the tube. The specimen thus obtained represents the contents (juice) of the fasting stomach. The tube is then removed and the patient is given a modified Ewald meal, consisting of half a shredded wheat biscuit soaked in 200 cc. of warm water. The tube is reinserted exactly an hour after the beginning of the meal, and another sample of gastric contents obtained. Both samples are analyzed for acidity (page 4); if free acidity is absent or very low, the histamine test is performed the next morning.

Alcohol Fractional Test. The method described is that of Dietrich and Shelby (8). A sample of gastric juice is obtained from the fasting child by the same procedure as in the preceding test. A soft nelaton catheter should be used, instead of the Jutte tube originally recommended. After obtaining the specimen, the tube is anchored to the child's cheek with adhesive tape, and 50 cc. of 7 per cent alcohol are administered by gavage. Ten minutes after the test meal a specimen is aspirated; 2 more specimens are obtained at 10 minute intervals, and all 3 are analyzed for acidity (see below). If none of the specimens show free acidity above 8–12 degrees, the histamine test is performed the next morning.

Histamine Fractional Test. The method described is that of Carnot, Koskowski, and Libert (9), as modified by Cutter (10). The test is carried out in the morning on a fasting stomach (infants up to 10 days, 4 hour fast; others, 8 hour fast). In infants, a soft French catheter is introduced gently through the mouth; in older children, through the nostril if tube's size permits. When the tube is in proper position, a specimen is aspirated with a 20–50 cc. syringe, and the tube is anchored to the child's cheek with adhesive tape. Then 0.01 mg. histamine (0.019 mg. histamine phosphate) per kilogram of body weight is injected subcutaneously, the maximum for children being 0.25 mg. A total of 4 specimens for 4 successive 10 minute periods is collected, and each specimen transferred to a separate test tube. In young infants suction should be applied only intermittently (every 3–5 minutes) to obtain the 4 samples. Each specimen is analyzed for acidity (see below). If free hydrochloric acid is absent in any one of the 4 samples, the combined histamine–neutral red test will enhance the significance of the results.

Histamine–Neutral Red Test. The method described is that of Glaessner and Wittgenstein (11). The procedure is the same as for the histamine test, but in addition 1–2 cc. of a sterilized solution of neutral (vital) red containing 40 mg. dye in 3 cc. distilled water are injected intramuscularly immediately following the histamine injection. A specimen of gastric juice is aspirated every 15 minutes for 2 hours, and examined for the presence of the dye.

Determination of Free and Total Acidity. The volume of the specimen is measured. The specimen is then filtered through a fivefold thickness of gauze or cheesecloth, and 4–5 cc. of the filtered specimen are measured into a white evaporating dish. To this 1–3 drops of Toepfer's reagent (0.5 Gm. dimethylaminoazobenzene in 100 cc. of 95 per cent ethyl alcohol) are added. An intense red color develops if free hydrochloric acid is present. From a graduated buret 0.1 *N* sodium hydroxide is slowly added to the mixture with constant stirring until the color turns canary yellow. The cubic centimeters of sodium hydroxide used are noted. This figure is used to calculate the cubic centimeters of 0.1 *N* sodium hydroxide which will neutralize 100 cc. of the gastric contents. The figure thus obtained represents the acidity per cent of free hydrochloric acid, or degrees of free acidity.

To the same mixture are now added 1–3 drops 1 per cent phenolphthalein in 95 per cent alcohol, and then, drop by drop, 0.1 *N* sodium hydroxide until a pink to red color develops and remains. The cubic centimeters of sodium hydroxide used for this titration are noted; the total cubic centimeters of 0.1 *N* sodium hydroxide used in the two titrations, corrected for 100 cc. of gastric contents, represent the acidity per cent of total acidity, or degrees of total acidity.

If the volume of filtered specimen is less than 2.5 cc., the titration is carried out with 0.01 *N* sodium hydroxide. The results, however, are expressed as cubic centimeters of 0.1 *N* sodium hydroxide.

Example. Titration of 4 cc. of filtered gastric contents may require:

(*a*) 1.3 cc. 0.1 *N* sodium hydroxide with Toepfer's reagent as indicator.
Acidity per cent of free acidity $= 1.3 \times 100/4 = 32.5$ degrees

(*b*) 1.8 cc. 0.1 *N* sodium hydroxide with phenolphthalein as indicator.
Acidity per cent of total acidity $= (1.3 + 1.8) \times 100/4 = 77.5$ degrees

The gastric contents aspirated after a test meal consist of pure gastric juice diluted by the ingested meal. Gastric acidity, as analyzed in these contents, has been commonly reported without taking into account such dilution. Since the histamine test now permits analysis of pure juice, its composition no longer need be calculated from the results after a test meal.

Determination of Hydrogen Ion Concentration. About 10 cc. (or quantity available) of gastric specimen are filtered through a Whatman filter No. 42 and a clear filtrate obtained. Dialysis through a collodion membrane has also been recommended. The pH in the filtrate or dialysate is determined by one of the colorimetric methods (12,13).

INTERPRETATION

Normally, gastric acidity increases steadily from infancy through childhood, with adult values reached by the age of 20. The smaller the subdivisions of the age groups for which normal values are available, the more accurately can the analytic results be computed. But since fluctuations even within the various age groups are great, all average normal values (Tables 2–4) should be used with caution,

TABLE 2
Normal Gastric Acidity after Ewald Test Meal

Age, years*	Free acidity, degrees		Total acidity, degrees	
	Range†	Mean	Range	Mean
1–2 (14)	—	—	10–55	26.0
2–4 (14)	—	—	28–77	43.9
2–10 (15)	—	12.5	—	48.5
4–12 (8)	0–47	22.2	20–73	43.2
1–4 (16)	M 0–39	14.7	—	—
	F 0–59	12.0	—	—
5–9 (16)	M 0–59	27.2	—	—
	F 0–49	24.6	—	—
10–14 (16)	M 1–59	28.5	—	—
	F 1–59	22.7	—	—
15–19 (16)	M 20–99	50.7	—	—
	F 0–69	32.1	—	—
20 (16)	M 1–99	49.0	20–110	65.8

* Figures in parentheses are reference numbers.
† M, male. F, female.

TABLE 3
Gastric Acidity in Normal Children, Four to Twelve Years Old, after Alcohol Test Meal*

Gastric juice, time taken	Free acidity, degrees		Total acidity, degrees	
	Range	Average	Range	Average
Fasting..........	0–48	22.5	5–70	41.6
After 10 min.....	4–80	32.3	8–106	51.4
After 20 min.....	11–92	45.6	25–116	65.0
After 30 min.....	8–91	50.3	22–113	71.0

* After Dietrich and Shelby (8).

TABLE 4
Gastric Acidity in Normal Children after Histamine Injection

Age*	Gastric juice, sample analyzed	Free acidity, degrees		Total acidity, degrees	
		Range	Average	Range	Average
4–10 days (10)	†	0–20	8.7	15–40	27.2
17–34 days (10)	†	0–13	3.4	5–33	14.9
2.5–5 mos. (10)	†	9–59	22.3	22.5–71	37.2
6–12 mos. (10)	†	10–80	30.5	22.5–105	46.2
1–4 yrs. (10)	†	15–95	51.2	26–106	65.1
4–12 yrs. (8)	10 min.	19–117	68.7	39–140	91.9
	20 min.	57–113	81.7	80–131	106.2
	30 min.	52–110	89.0	71–145	114.1
	40 min.	51–113	76.7	70–205	100.8
20 yrs. (17)	†	—	—	80–142	123.0

* Figures in parentheses are reference numbers.
† Sample with highest acidity.

and unusually low or high results should be checked by repeating the test.

Table 2 lists normal values for free and total acidity obtained after ingestion of the Ewald test meal. Unfortunately, the figures for the younger children represent averages of measurements of several age groups, so that the rapid change in normal values which occurs between 2 and 3 years is not revealed by these data. In Table 3 will be found the normal response of all but the youngest aged children to the alcohol test meal.

The values for free and total acidity in normal children after stimulation with histamine, as shown in Table 4, represent the most significant measurements of the physiologic development of gastric secretory capacity during infancy and childhood, and may be considered completely reliable. In the histamine test one may record the gastric acidity of each 10 minute sample or only of the one with the highest acidity (usually the last sample).

Normal *p*H in the gastric contents varies with the test meal and the child's age (Fig. 1). Thus, after milk feedings, *p*H is the result of acid secretion, the acidity of the milk formula, and of the formula's buffering power (19a).

The response to the histamine–neutral red test is considered to be positive when the dye appears in at least one specimen. Normally, excretion of the dye starts within 15 minutes of the injection, continuing for an hour or more, and it is remarkably constant on different occasions in the same individual (19b). Total absence of dye excretion indicates that the gastric mucosa is incapable of secreting hydrochloric acid. According to Gillman (19b), "the function of gastric excretion of neutral red is the last activity that the stomach loses in the course of mucosal atrophy."

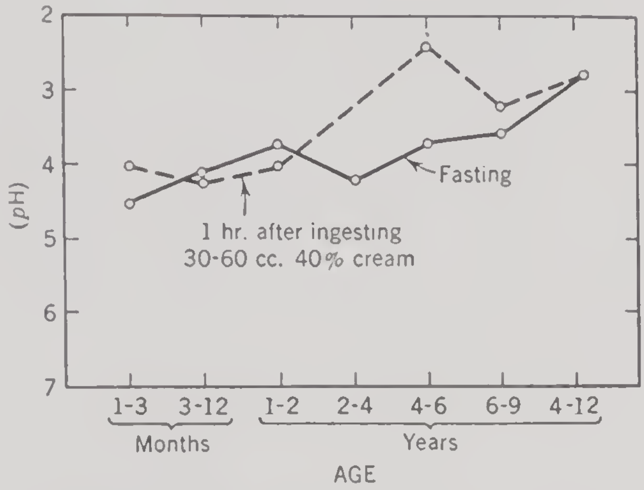

Fig. 1. Average hydrogen ion concentration of gastric juice in children, in fasting state and after test meal. Based on data of Klumpp and Neale **(18)**.

In *hyperchlorhydria* the stomach contents, examined by either test method, give abnormally high values both of free and total acidity. A diagnosis of hyperacidity is indicated when the values are 50 per cent or more above the average normal.

In *hypochlorhydria* persistently low values of free acidity are obtained after either test, the figures showing a decrease of 40 per cent or more as compared with the average normal. Below 8 degrees in children up to 4 years, below 15 degrees in children 4 to 12 years, and below 20 degrees in adults justifies a diagnosis of hypoacidity.

In *apparent achlorhydria* there is absence of free hydrochloric acid after test meals, but a positive response to stimulation with histamine.

True achlorhydria may be diagnosed tentatively when test meals and histamine injection fail to elicit secretion of hydrochloric acid. For a positive diagnosis, however, the histamine neutral red test should be performed. Winkelstein (20) says: "If the dye appears and free acid does not, it signifies in our experience that the stomach is capable of secreting free acid, that the achlorhydria is a temporary or false one." Total suppression of neutral red excretion may be regarded as proof that the observed achlorhydria is "true."

Hyperacidity, as well as hypoacidity, seems to occur with the same frequency in infants and children as in adults. The wide range of normal values even includes values commonly considered as pathologic. Abnormally high or low gastric acidity in a child does not in itself indicate whether, as Alvarez (16) puts it, it is a disease about which we should become anxious or whether it is a symptomless peculiarity of the individual. Pediatric experience has proved that the second interpretation is the correct one in most instances.

Hyperacidity may occur in healthy children at any age as a sign of a constitutional imbalance of the autonomic nervous system. But the consistent relationship between hyperacidity and stomach or duodenal ulcers should be remembered even by pediatricians, since such ulcers occur in very young children not as infrequently as might be expected.

In children, hypoacidity seldom indicates definite pathology. When it occurs in a healthy child, constitutional factors may be the cause. In a sick child it may be a sign of acute or chronic disturbances of the intestinal tract, of celiac disease, or of Addison's disease, but it is not a specific sign for any of these conditions.

Anacidity is very rare during childhood, perhaps because conditions in which it very frequently occurs, e.g., pernicious anemia, carcinoma of the stomach, and cholecystitis, rarely affect children. No conclusions can be drawn from reports in the literature on a relatively high incidence of anacidity in healthy and sick children, since the figures are almost exclusively based on methods using test meals only.

INTESTINAL ABSORPTION TESTS

No satisfactory methods for measuring intestinal absorption have yet been devised. For clinical purposes, however, impaired absorption of certain foodstuffs may be inferred from the composition of stool and urine, and from results of oral loading or tolerance tests.

If other causative factors, such as impaired or absent digestive enzyme activity, re-excretion into the intestines, or bacterial action, can be ruled out, presence in the feces of undigested food residues or of a very high amount of a normal constituent may indicate faulty absorption. Chemical tests (e.g., fractional analysis of protein or fat derivatives) for determining the primary cause of abnormal stool content are laborious, and not always necessary since simple microscopic examination yields clinically important information as to the status of intestinal absorption. Thus, the increased fat content of the stool in celiac disease, established microscopically, points primarily to impaired absorption, for the activity of fat-splitting enzymes has been shown to be essentially intact in this disorder (page 11). In pancreatic deficiency, on the other hand, the presence of unchanged food proteins in the stool is the result of enzymic (tryptic) insufficiency; failure of these complex substances to be absorbed is not a sign of faulty intestinal absorption. For the technic of stool examination, see pages 20–22.

Increased permeability of the intestinal mucosa, i.e., faulty absorption, has long been considered the sole cause of the appearance of polysaccharides (e.g., lactose) in the urine of apparently healthy infants. Numerous observations suggest that intermediary metabolic processes are responsible, at least in part.

Blood tolerance curves obtained from oral loading tests always provide some information as to the rate at which the test substance is absorbed by the intestine and delivered into the blood stream. But other factors, such as rate of withdrawal from the blood (assimilation), renal threshold, and re-excretion into the intestine may decisively influence the curve. That is why such tests should be called "tolerance" or "loading" tests; the term "absorption test" is a misleading one unless it has been proved that absorption from the bowel is the only, or almost the only process responsible for the blood curve in a particular instance.

For the role played by absorption and assimilation in the various tolerance tests—dextrose, levulose, galactose, amino acid, vitamin A, vitamin C loading, and blood fat loading—see the tests in question.

PANCREATIC ENZYME ACTIVITY TESTS

The laboratory procedures for testing the digestive (enzymic) function of the pancreas which are of specific diagnostic value are (*a*) trypsin, amylase, and lipase assays in the duodenal juice; (*b*) the same analyses after stimulation with secretin; (*c*) determination of the activity of these enzymes in the serum; (*d*) examination of the stool for abnormal content of fat, nitrogen, and starch; (*e*) measurement of urinary enzyme excretion.

Acute pancreatic conditions (acute edema, acute necrosis, carcinoma of the pancreas) are so uncommon in children that one rarely needs to call on tests which, according to Pratt (21), "are most useful to detect such conditions in their early stage." Such tests include the ones listed above under *b*, *c*, and *e*. What the pediatrician needs mainly are diagnostic aids for recognizing *chronic* pancreatic disease, and in this the tests included in groups *a* and *d* are of specific value. These tests not only demonstrate the presence of pancreatic dysfunction, but also differentiate between the various disease entities forming the clinical picture of the celiac syndrome. Since the findings with regard to the activity of the pancreatic enzymes are typical for each of the known conditions (22), the assay of the enzymes in the duodenal juice is by far the most important diagnostic aid in pancreatic disease of infants and children. Stool tests are likewise indispensable. Table 5 classifies the findings by which a differential diagnosis may be made of "celiac" patients with pancreatic insufficiency.

TABLE 5
Enzymic Activity of Duodenal Juice and Composition of Stool in Celiac Syndrome

	Results obtained in	
Determinations	classic celiac disease (with or without steatorrhea)	cystic fibrosis of pancreas
Duodenal juice		
Tryptic activity	Normal	Absent or low
Diastatic activity	Absent or low	Absent or very low
Lipolytic activity	Normal	Very low
Volume	Normal	Small or very small
Viscosity	Normal	Very high
Stool		
Total fat content	Normal, increased, or very high	Increased or very high
Undigested starch content	Increased	Increased
Clinical intolerance to	Starch, or starch and fat	Fat and starch

According to Anderson (22,23a–b).

At the present time Anderson (23b) recognizes only two groups in the celiac syndrome—uncomplicated celiac disease and fibrocystic disease of the pancreas. All other conditions answering the clinical description of the celiac syndrome fail to show well-defined enzymic deficiencies in the pancreatic juice, and must be differentiated on clinical grounds.

Klumpp and Neale (18) have established the normal standards of tryptic, amylolytic, and lipolytic activity of duodenal juice in children, and Anderson (22) and Shwachman, Farber, and Maddock (24) have confirmed them. One thus has the basis for clinical interpretation of the results of determinations for these enzymes (25).

Brief comment on the vitamin A absorption test and its diagnostic significance in pancreatic disorders of children is in order at this point. An abnormally low rate of vitamin A absorption is regularly associated with the celiac syndrome (page 258), and it may well be considered as one of the diagnostic criteria (26); however, differentiation of the various disease entities comprising the syndrome is not aided even when very low vitamin A absorption is established. While a lowered rate of absorption strongly suggests the presence of the celiac syndrome, it does not indicate whether pancreatic insufficiency is its cause. This can only be established by determination of the activity of the pancreatic enzymes (27). If the absorption of vitamin A is normal, the presence of any disease of the celiac syndrome is practically precluded.

Assay for Pancreatic Enzymes in Duodenal Juice

Duodenal Drainage. The simplest device for obtaining duodenal juice from children is a smooth rubber tube 3 to 5 mm. in diameter and about 50 cm. (4 ft.) long, or French tubes (No. 10 for infants, No. 12 for children). Anderson (22) supplements the French catheter tubing with a solid, dumbbell-shaped metal tip, less than 1 cm. long and 4 mm. in diameter, which she ties with silk into the distal end of the tube. Whatever the tube used, it must contain 3 or 4 openings in the distal 5 cm. A bakelite or glass adapter may be attached to any of these tubes, connecting it with another rubber tube into which a 20 cc. Luer syringe, mouthpiece, or other suction device is fitted at the other end. In addition to the fluoroscopic observation, a convenient aid to judging the tube's progress are marks on the tube corresponding to the distance between a child's lip and the fundus of the stomach. The distances should be measured from the tip of the tube, and according to Cutter (10) the average distances are 22 cm. in the newborn, 26 cm. at 6 months, 30 cm. at 1 year, 35 cm. at 2 years, increasing gradually to 40 cm. or more in older children.

The tube is passed through the mouth in infants under 6 months, through the nostril in children over 6 months. Gastric contents, if there are any, are aspirated as soon as the tube reaches the fundus. The tip of the tube is then guided, under fluoroscopic control, into the pyloric antrum and given a chance to slip into the duodenum. Forceful manipulation of the tube against the closed pyloric ring should be avoided, as this may cause the tube to coil and prevent drainage. Passage of the tube through the pyloric channel is accomplished in a few minutes to half an hour (rarely longer), during which interval water may be offered, but no other fluid (28). The position of tube should be determined fluoroscopically; if the tip is not in the duodenum, it should be replaced in the pyloric antrum and given another chance to pass through the channel. When the tip is finally in the duodenum and has passed 3 to 4 cm. beyond the pylorus, i.e., to the middle third of the duodenum, the tube is anchored to the face with adhesive tape. The juice should be tested with litmus paper as soon as the flow is established, and testing continued until an alkaline reaction is obtained. Collection of the specimen is then started, the child remaining in bed or on the examination table during the procedure.

The juice may be obtained by gravity, but preferably it should be drawn by aspiration. This may be done by means of (*a*) a 20 cc. Luer syringe fitted into the distal end of the tube; (*b*) constant suction by a device identical to the one used for drainage of pleural empyema; or (*c*) aspiration through a mouthpiece. A pear-shaped glass bulb (Fig. 2), placed about the middle of the tube connecting adapter with suction device or mouthpiece when method *b* or *c* is used, or even with method *a*, is the device used for collecting the juice. The fluid is collected in 10 to 30 minute periods, their number depending on the rate of flow, and the specimens are kept in the refrigerator until analyzed. Normally, up to 15 cc. of juice per

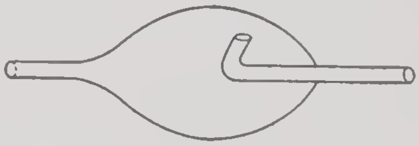
Fig. 2. Glass container for collecting duodenal juice.

hour are obtained from infants up to 1 year, and as much as 50 cc. in older children, but only about 10 cc. are required for a complete analysis.

TRYPSIN TEST

The method used is that of Fermi, as modified by Anderson and Early (29). It is based on the principle that gelatin is liquefied by trypsin (pancreatic protease). Tryptic activity of duodenal juice is therefore measured by determining the amount of duodenal juice which will liquefy a given volume of gelatin solution of constant composition.

PROCEDURE

Gelatin Substrate. Place 18.75 Gm. of Knox gelatin in a 50 cc. beaker and gradually stir in about 40 cc. of cold, distilled water until the mixture becomes a smooth and semiliquid paste. Transfer through a small funnel into a 200 cc. volumetric flask, and wash beaker and funnel with about 100 cc. of water. Heat the flask on a water bath, with occasional shaking, until the solution is clear, put aside to cool, and make up to volume with distilled water. Replace on the water bath until the gelatin has completely melted. Shake until the contents appear homogeneous, transfer the solution to a stock bottle, and add a small amount of thymol. If stored in a refrigerator, the solution keeps for at least 6 weeks, but it must be melted before use by being placed in the incubator for an hour at 37 C.

For the test 20 Wassermann tubes are set in a rack in pairs and labeled from 1 to 10. In a 10 cc. volumetric flask are placed 2 cc. of the melted substrate and made up to volume with a 5 per cent solution of sodium bicarbonate, making a 1:10 dilution. In a second flask, 1 cc. of the 1:10 dilution is placed and made up to volume, resulting in a 1:100 dilution. Using a 1 cc. Mohr pipet, 0.125 cc. of the 1:100 solution and 1.875 cc. of 5 per cent sodium bicarbonate solution are measured into each of the first pair of tubes, making a total volume of 4 cc. in each tube, of which 0.00125 cc. is duodenal juice. The remaining 9 sets of tubes are set up in a similar manner. Each of the second pair receives 0.25 cc. of the 1:100 dilution; the third and fourth pair, 0.5 and 1.0 cc. of the 1:100 dilution; the fifth, sixth, seventh, and eighth, 0.2, 0.4, 0.8, and 1.6 cc. of the 1:10 dilution; the ninth pair, 0.32 cc. of the undiluted juice in a total volume of 4 cc.; and the tenth or control pair of tubes contains no duodenal juice.

Each tube is covered with the thumb and inverted several times to insure thorough mixing of the contents. The rack of tubes is then placed in the incubator, left for an hour at 37 C., placed in the refrigerator for 24 hours, then removed and read immediately. The lowest dilution of duodenal juice which has liquefied the gelatin in one or both tubes is noted, and the result given in cubic centimeters of duodenal juice present in the dilution in question.

Tryptic activity is customarily expressed in terms of units, as measured by a viscometric method, a unit of trypsin being defined as the amount of enzyme required to reduce the initial viscosity of a starch solution–enzyme mixture by 20 per cent in 1 hour. In Table 6 the results of the Fermi method are converted into units of trypsin.

TABLE 6
Tryptic Activity, Correlation of Results Obtained by Modified Fermi and by Viscometric Methods

Fermi method, cc. of duodenal juice used	Viscometric method, units of trypsin per cc.
0.0025	400
0.005	250
0.01	130
0.02	75
0.04	40
0.08	25
0.16	15

From Shwachman, Farber, and Maddock (24).

INTERPRETATION

Normally, the minimal amount of duodenal juice which gives a positive trypsin result varies between 0.0025 and 0.04 cc. (400 to 40 trysin units per cubic centimeter), depending on the child's age. Table 7 gives the mean values for normal children. Values of 0.1 to 0.3 cc., or less than 20 trypsin units per cubic centimeter, or complete absence of trypsin activity, signify pancreatic insufficiency and indicate a diagnosis of cystic fibrosis.

LIPASE TEST

The method used is that of Willstätter, Waldschmidt-Leitz, and Memmen (30), as modified by Shwachman, Farber, and Maddock (24). The test is based on the principle that fatty acids are liberated from olive oil by the activity of the steapsin in pancreatic juice. The lipolytic activity of duodenal juice is proportional to the amount of fatty acids, determined by titration, freed by a volume unit of juice.

PROCEDURE

Reagents.

(*1*) Olive oil, Laco brand, saponification number 193.
(*2*) Aminoacetic acid buffer, *p*H 8.9. Make a solution of 9 parts glycocoll solution (7.505 Gm. glycocoll, 5.85 Gm. sodium chloride per liter) and 1 part 0.1 *N* sodium hydroxide at 18–20 C.
(*3*) Freshly prepared 3 per cent egg albumin solution.
(*4*) 2 per cent calcium chloride solution.
(*5*) Alcohol–ether mixture. Mix 4 parts of 95 per cent ethyl alcohol and 1 part ether.
(*6*) 0.1 *N* alcoholic potassium hydroxide.

To 2.5 Gm. olive oil in a glass-stoppered Erlenmeyer flask are added 2 cc. aminoacetic buffer, 0.5 cc. calcium chloride solution, 0.5 cc. egg albumin solution, and 10 cc. of a 1:10 dilution of duodenal juice. The flask is placed in a water bath at 37.5 C., and shaken vigorously for 3 minutes, then left at this temperature for 1 hour (incubation period of 63 minutes), after which 35 cc. of the alcohol–ether mixture are added to terminate the reaction. Determinations are made in duplicate, with a third determination serving as control, in which enzymic activity is inhibited by adding the alcohol–ether mixture immediately after the duodenal juice.

The liberated fatty acids are titrated against 0.1 *N* alcoholic potassium hydroxide, thymol blue being used as indicator. The cubic centimeters of potassium hydroxide required to turn the control mixture distinctly blue varies between 3.3 and 3.6 cc. in different samples of duodenal juice. This control value is subtracted from the cubic centimeters of potassium hydroxide required to neutralize the fatty acids in the sample tubes.

A lipase unit is defined as the amount of lipase which, under given conditions, will split 24 per cent of 2.5 Gm. of olive oil. One unit equals 20.7 cc. of 0.1 *N* potassium hydroxide solution.

Calculation.

$$\text{Units per 100 cc. juice} = (\text{cc. } 0.1\ N\ \text{KOH}/20.7) \times 100$$

INTERPRETATION

The reliability of the test is impaired by "considerable variation of lipolytic activity in different specimens of duodenal juice obtained either during one collection period or at a later date from the same patient" (25). Lipolytic activity in normal children varies widely, ranging from 38 to 100 lipase units per 100 cc. of duodenal juice (Table 7). In chronic idiopathic steatorrhea, the classic celiac

TABLE 7
Activity of Pancreatic Enzymes in Pancreatic Juice in Children: Normal and in Celiac Syndrome

Condition and age	Trypsin, units/cc., mean	Amylase, units/cc., mean	Lipase, units/cc., mean
Normal			
0–2 mos.	137.6	4.3	21.2
2–6 mos.	138.8	25.3	26.6
6–12 mos.	250.5	113.9	34.6
1–2 yrs.	262.0	117.4	18.8
2–5 yrs.	195.0	243.9	19.6
Idiopathic steatorrhea			
16 mos.	185.0	156.0	20.8
3 yrs.	220.0	364.0	18.0
Starch intolerance			
1–2 yrs.	188.0	61.8	20.0
Cystic fibrosis of pancreas			
1 mo.	1	0	0
5 mos.	0	44	0
10 mos.	0	0	0
18 mos.	0	0	3
2.5 yrs.	1	0	0

After Anderson (22).

disease, activity of 40 to 60 units is the rule. Values of 0 to 10 units are typical of pancreatic fibrosis.

DIASTASE (AMYLASE) TEST

The method described is that of Thompson, Tennant, and Wiese (31). The test is based on the principle that when pancreatic juice is allowed to act upon a buffered starch solution the viscosity of the mixture decreases progressively, in proportion to the rate of starch digestion. The initial viscosity of the substrate–juice mixture is reduced rapidly if the diastatic activity of the juice is high and vice versa.

PROCEDURE

To prepare the starch substrate, add 15 cc. of 1 *M* sodium acetate solution and 45 cc. distilled water to 5 Gm. of soluble starch. The solution is cleared by gentle heating, autoclaved for 20 minutes at a pressure of 18 pounds (8 Kg.), and 5 cc. of 1 *M* acetic acid are added. The mixture is transferred to a 100 cc. volumetric flask containing 10 cc. of 10 per cent calcium chloride in 0.15 *M* sodium acetate and 0.05 *M* acetic acid, and water added to volume. The solution is placed in an oven, and the temperature of the solution maintained just above 50 C. while it is being filtered through a Whatman filter No. 42. The clear filtrate is adjusted to *p*H 6.9. If stored in the refrigerator, it keeps for about a week.

Ostwald type 5 cc. viscometer tubes are used for the test. The starch substrate is placed in the incubator for 1 hour at 37.5 C. Several lots of 20 cc. each are then measured out and 4 cc. of duodenal juice dilution are added to each lot to form the digestion mixture, a 1:10 dilution of juice for infants under 4 months, a 1:100 dilution for children over 4 months. The digestion mixture is kept at a constant 37.5 C. in a water bath or incubator, and portions of the mixture are transferred into viscometer tubes, beginning 3 minutes after the addition of the duodenal juice and continued at 5 minute intervals until the initial viscosity is reduced about 20 per cent. With the above dilutions this occurs in 20 to 60 minutes. In estimating viscosity, the outflow time between marks is observed with a calibrated stop watch.

When kept at room temperature for 24 hours before analysis, duodenal juice loses about 50 per cent of its activity. In the refrigerator, however, the loss is considerably smaller.

An amylase unit is arbitrarily defined as the amount of enzyme required to reduce the initial viscosity of a starch solution–enzyme mixture by 20 per cent in 1 hour.

Calculation.

$$\text{Units per cc. juice} = 60/(TV)$$

where T is time in minutes required to reduce viscosity 20 per cent, and V is the volume of duodenal juice (enzyme solution) contained in the dilution added to the substrate.

INTERPRETATION

Normally, diastatic activity is low in infants up to 6 months, usually below 50 units per cubic centimeter, and sometimes 0 to 10 units. Above that age, normal activity ranges between 100 and 250 units (Table 7). Low figures, therefore, have little significance in young infants. In older infants and children, low activity, i.e., about 50 units or less, is found in starch intolerance of uncomplicated celiac disease. In the presence of cystic fibrosis of the pancreas, the test will show extreme reduction or absence of activity, irrespective of age.

Admixture of gastric juice to the duodenal contents in the course of duodenal drainage frequently leads to erroneous results; it causes dilution and may introduce amylase from saliva. In order to reduce such errors in evaluating test results, Anderson (23b) recommends that the ratio of diastase concentration to trypsin concentration expressed in viscosimetric units be used. According to this author, changes in the ratio are more significant than deviations in the absolute concentration of diastase. The normal ratio of diastase to trypsin during the second half of the first year is of the order of 50 per cent, while in children over 1 year old it ranges between 50 and 150 per cent. The value of this ratio which marks the dividing line between the normal and abnormal, is taken as 20 per cent for children between 6 and 12 months of age, and 40 per cent for those who are older.

SECRETIN TEST

Secretin is a hormone-like substance. It is produced by the mucosa of the duodenum and small intestine upon contact with the acid chyme and is absorbed into the blood stream. Its action stimulates pancreatic secretion. A crystalline, powdered product, first isolated by Hammarsten (32) is available commercially.

Observations on normal adults (33,34) have shown that intravenous injection of secretin causes a more or less copious flow of normal pancreatic juice. The response of adults with chronic pancreatic disease is abnormal: rate of flow is not accelerated appreciably and there is decreased enzymic activity. Furthermore, in one report (35) attention is called to the abnormally low sodium bicarbonate content of "secretin juices" in patients with chronic pancreatic disease.

Knowledge of children's response to the secretin test is limited. From observations by Maddock *et al.* (36), it would seem that the response in children with the celiac syndrome is abnormal, as compared to that of normal children. However, it has not yet been established whether the test is clinically valuable for the differentiation of the celiac syndrome.

BLOOD TESTS

SERUM DIASTASE TEST

As yet, this test is of no particular value in diagnosing chronic pancreatic disease in children. Acute pancreatitis is sometimes more easily recognized if the urine rather than the serum is examined for increased diastase concentration.

SERUM LIPASE TEST

In adults this test is considered "a trustworthy means for detecting most cases of acute pancreatic disease" (21). No evidence, however, is available as to its diagnostic value in children with chronic pancreatic deficiency.

STOOL TESTS

Such tests are of diagnostic worth only if the child has received a diet normal for its age for a few days preceding the examination. The tests consist of microscopic examination for fat, starch, and muscle fibers, and chemical analysis for total fat.

FAT TEST (MICROSCOPIC)

A drop of stool, drawn from within the specimen, and 2 drops of 1 per cent scarlet red in absolute alcohol are mixed on a slide by stirring, and then mixed with 1 drop of saline solution. A cover slip is placed over the slide and the specimen is ready for examination.

The fat present may appear as round droplets stained red, or as clumps of spindle-shaped crystals stained faint orange or not stained at all. An excessive amount of fat droplets or crystals is characteristic of any form of steatorrhea. Anderson (37) states that the microscopic examination for fecal fat gives a quantitative estimate of fat excretion which is highly reliable when microscopic gradings are 0 or plus 3 to 4, but that intermediate gradings cannot be interpreted accurately.

The method is useful for screening out patients without steatorrhea, but chemical analysis is required for more accurate determination of the fecal fat.

STARCH TEST (MICROSCOPIC)

A drop of stool not more than a few hours old is mixed on a slide with 1 drop of *N* ammonium chloride, and then with 1 drop of 3.5 per cent solution of iodine in 5 per cent potassium iodide. Starch granules stain blue, but the free granules must be distinguished from those enclosed in vegetable cells. Normally, free, extracellular granules are infrequent, but in stools from children with starch intolerance (celiac disease, pancreatic fibrosis) such granules are present in large numbers (Table 5). Intracellular starch granules may be ignored except when they occur in excessive amounts.

MUSCLE FIBER (CREATORRHEA) TEST (MICROSCOPIC)

It is not yet certain whether the presence of a considerable number of undigested muscle fibers in the stools of children with chronic nutritional disturbances (various forms of celiac syndrome) is indicative of diminished pancreatic function. Food may move so rapidly through the small intestine in many of these patients that the presence of muscle fibers might be the result of mechanical factors rather than of digestive enzymic failure.

ANALYSIS FOR TOTAL FAT

The method outlined here is that of Reiner (38). The child is placed on a normal diet for 1 to 2 days before the test. Stools are then collected for 48 to 72 hours and kept in closed glass containers in the refrigerator until the analysis is carried out.

The total collected stool is dried on a water bath, and mixed and ground to a powder with mortar and pestle. In a 100 cc. glass-stoppered, gradulated cylinder are placed 1 Gm. dried feces, 20–30 cc. water, and 2 cc. concentrated hydrochloric acid, and the whole shaken for 5 minutes. Then 20 cc. ethyl ether are added to the mixture and the contents shaken for 5 minutes, after which 20 cc. 95 per cent alcohol are added, and the mixture again shaken for 5 minutes. The cylinder is set aside for 10–15 minutes, and the supernatant ether layer is then pipetted into an evaporating dish. Another 20 cc. ethyl ether is added to mixture in cylinder, shaken for 5 minutes, and set aside until the mixture settles, after which the ether layer is again removed into the evaporating dish. This extraction is repeated three more times. The total ether washings in the dish are evaporated and the residue of fat is dissolved in 5–10 cc. of petroleum ether (b.p. 40–60 C.). This solution is filtered into a small, weighed dish which is placed in a water bath to evaporate the petroleum ether. The residue in the dish is dried, and the dish with its contents weighed to constant weight. This weight, minus the weight of the empty dish, represents the total fat, and is expressed as per cent of the dried matter of the stool specimen.

The normal fat content in children and adults varies between 10 and 22 per cent of the weight of dried stool. The fat content in celiac disease (idiopathic steatorrhea) is between 31 and 44 per cent, and in pancreatic fibrosis it is even higher, ranging from 30 to 80 per cent. But in other forms of the celiac syndrome the fat content of the stool is in the main normal (Table 5).

For the fractional analysis of the stool fat, Anderson recommends her modification of the Sperry method (37).

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CHAPTER II

Liver Function Tests

The manifold functional activities of the liver fall into the groups of excretory, secretory, and metabolic functions (page 26). Liver damage may affect one or a number of these functions; when several activities are impaired, it is improbable that all will be injured to the same degree.

Many methods have been devised for determining the functional capacity of the liver. Each test deals with a specific function, and has its own clinical significance and limitations. Since no one test adequately measures several or all of the hepatic functions, ampler information will obviously be provided by a combination of tests. The more carefully the test methods are selected, the more valuable will be the results they yield.

The results of hepatic function tests must be interpreted with great caution in infants and young children. Early in life the liver is an extremely sensitive organ, easily affected by common diseases, such as fever, intercurrent infections, or nutritional disorders. Hepatic involvement in such cases is not of serious import except when the impairment is marked, and the management of such secondary hepatic disorders in children does not require function tests to the same extent as in adults.

Some of the tests used for adults cannot as yet be considered reliable diagnostic procedures for children. In the discussion that follows, the selection of tests, their adaptation for use in children, and the interpretation of results will be considered. The author found valuable guidance in similar presentations by other workers for use in adults (1–5).

CHOICE OF TEST

The following tests have been selected as the most valuable in the clinical study of liver function in children, grouped according to the functions they attempt to explore.

- Excretion of bile pigments
  - Blood tests
    - Van den Bergh test (qualitative) (page 30)
    - Van den Bergh test (quantitative) (page 31)
    - Icterus index (page 31)
    - Serum bilirubin determination (page 33)
  - Urine tests
    - Examination for bilirubin (page 36)
    - Examination for urobilinogen (page 37)
- Loading tests
  - Bromsulfalein excretion test (page 38)
  - Bilirubin excretion test (page 41)
- Carbohydrate metabolism
  - Dextrose tolerance test (page 103)
  - Levulose tolerance test (page 112)
  - Galactose tolerance test (page 115)
  - Blood glycogen determination (page 127)
- Fat metabolism
  - Total cholesterol in serum (page 137)
  - Cholesterol partition in serum (pages 41,137)
- Formation of serum proteins
  - Albumin determination (page 159)
  - Albumin-globulin ratio (page 163)
  - Cephalin-cholesterol flocculation test (page 43)
  - Takata-Ara test (page 44)
  - Colloidal gold reaction (page 45)
  - Thymol turbidity test (page 47)
  - Prothrombin time determination (page 49)
  - Prothrombin time response to vitamin K (page 53)
- Phosphatase formation
  - Alkaline phosphatase determination in serum (page 211)
- Detoxification
  - Hippuric acid test (page 54)

The proper choice of test depends on the general nature of a given pathologic condition. In choosing between the various available methods, the physician must decide upon the one which he believes will best answer the specific question raised by the clinical features of the disease. Perhaps the most significant clinical criterion for the selection of tests is the presence or absence of jaundice.

If Jaundice Is Present. Differentiation between obstructive and parenchymatous jaundice by means of tests which will show changes in the formation and secretion of bile is the first step. These tests are van den Bergh's test, urinalysis for bile and bile derivatives, the bromsulfalein test, phosphatase determination, and serum cholesterol determination. In over half of the cases, the diagnosis of obstructive jaundice can be made by analyzing the urine for bilirubin and urobilinogen (5), and one of the blood tests which reveal bile retention will furnish supporting evidence.

Hepatic or parenchymatous jaundice is revealed when the appropriate test demonstrates that the specific metabolic liver function is weakened. Theoretically, this group of tests should give negative results in all cases of extrahepatic, obstructive jaundice, and positive results in all forms of hepatitis or other injury to liver cells. Actually, however, a small degree of liver damage and slightly impaired function also occurs in obstructive jaundice after a certain length of time. The less sensitive tests are therefore more useful in differentiating between obstructive and parenchymatous jaundice. The levulose tolerance test, the hippuric acid test, and determination of the albumin–globulin ratio give positive results only in the presence of the more severe hepatocellular injuries. If jaundice due to extrahepatic obstruction persists for weeks, as, for instance, in congenital atresia of the bile ducts, there is progressive damage to the liver cells and functional tests disclosing such damage become strongly positive. In this stage such tests are useless in distinguishing hepatogenous from obstructive jaundice.

When extrahepatic obstruction can be ruled out as the cause of the jaundice and functional tests reveal the absence of intrahepatic injury, a hemolytic genesis is the only explanation left. In all probability, a definite answer will be provided by the urinalysis for bilirubin and urobilinogen and by van den Bergh's test (see Table 9, p. 37). Two additional tests—determination of phosphatase and cholesterol partition in the blood—reliably corroborate the findings of the above tests. The hemolytic character of the jaundice is almost definitely established if both tests yield normal results. Another means of differentiation, reported by Reiner and Weiner (6), consists in determining the ratio of the icteric indices obtained by the two methods—the water method and the acetone method.

In summary, tests on jaundiced children are primarily important in making the differential diagnosis of uncomplicated obstructive jaundice, uncomplicated parenchymatous jaundice, and hemolytic jaundice. The difficulties inherent in functional testing should always be kept in mind when obstructive jaundice is complicated by secondary hepatic insufficiency. Fortunately, clinical necessity for such strict differentiation is exceptional in children. Results of liver function tests are only rarely needed as criteria for surgical intervention in children. This is in marked contrast to the situation in adults with such conditions as cancer of the pancreas or biliary ducts, where indications for surgery depend on proof of potential hepatic capacity. In the majority of jaundiced children, therefore, function tests shed light on the pathologic condition, give a fair estimate of the extent of liver damage, and provide some support for prognosis.

Tests in Absence of Jaundice. One group of diseases of the liver unassociated with jaundice is the one in which hepatomegaly is present. This group includes Banti's disease, cirrhosis, pseudocirrhosis, fatty degeneration, lues, and glycogen disease. Since it is the glycogenic capacity of the liver which primarily suffers in these conditions, the various sugar tolerance tests, as well as the adrenalin test for sugar mobilization, are the most useful tests. Determination of the blood content of glycogen may also be helpful, when glycogen disease is suspected. The most sensitive tests in this group are the levulose and galactose tolerance tests, since they require stereo-isomer transformations and thereby exact a greater performance from the liver than the glucose tolerance test.

Patients with abnormal blood clotting properties are another group of nonjaundiced patients with potential impairment of liver function. Liver disease is a possible cause of coagulation defects; fibrinogen and prothrombin determinations will help to establish whether it is hepatic insufficiency that is the responsible etiologic factor.

When involvement of liver function is suspected in a nonjaundiced patient, with no clinical signs or symptoms pointing to a specific function or group of functions at fault, the presence of latent icterus should be considered first, and the icterus index, bilirubin in serum, or bile derivatives in urine be determined. Secondly, it must be established whether hepatocellular damage has taken place at all. This can be done by the bromsulfalein and hippuric acid tests and by such highly sensitive procedures as the Takata-Ara, cephalin–cholesterol flocculation. gold sol, and thymol turbidity tests. The latter group of serum flocculation tests have gained wide acceptance as most accurate means of detecting and appraising functional liver damage. The latest additions to this group are the thymol flocculation and zinc sulfate tests. Although based on the same principle and closely related in their clinical significance, these serum flocculation tests do not always give identical results when applied to the same patient. Each of the tests intimates a specific abnormality in the pattern of serum proteins. Liver function may appear normal when judged by one test of this group, but deficient when tested by a second one. The serum flocculation tests for liver function do not substitute for but supplement each other. Thus, the performance of at least two tests, for example, the cephalin–flocculation and the thymol turbidity tests, will greatly increase the diagnostic value of either one.

Two of the tests for hepatic insufficiency—the serum phosphatase activity and the galactose tolerance—have an entirely different significance when used to assess thyroid function. Reduced serum phosphatase activity in hypothyroid patients is understood to be the result of impaired osteoblastic activity rather than of bile retention, while an abnormally low galactose tolerance in hyperthyroid patients is at least partly the result of a failure of intestinal absorption.

It seems appropriate to mention here some observations (7) that have been made on patients with hepatolenticular degeneration (Wilson's disease). In some cases, diagnosis can be made in the initial stage of this progressive disease, when definite neurologic findings are still absent, by demonstrating an impairment of liver function. The bilirubin clearance test, prothrombin test, and the colloidal gold test have been reported as being particularly valuable in this condition.

In conclusion one might quote the advice of Ivy and Roth (5): "Classify the tests according to the diagnostic questions to be answered. Select a few tests and learn them and their limitations well."

TEST METHODS

VAN DEN BERGH'S TEST

The test (8) is based on the principle that bilirubin may be present in the serum in various forms or types. The differences between them are presumed to depend on whether or not the pigment has passed through the liver cells, the chemical and physical properties of bilirubin apparently being altered in the liver. However, the exact nature of these changes is still open to question. Ample evidence supports the belief that all bilirubin in the blood is attached to albumin (9), and that the main difference between "direct" and "indirect" bilirubin lies in the bond linking them to albumin fractions. The one fact definitely established is that the two types of bilirubin do not react in the same way to Ehrlich's reagent.

PROCEDURE

Reagent. Prepare Ehrlich's diazo reagent immediately before use by adding 0.3 cc. of a 0.5 per cent solution of sodium nitrate to 10 cc. of a solution composed of 0.1 Gm. sulfanilic acid, 1.5 cc. concentrated hydrochloric acid, and water up to 100 cc. The sodium nitrate solution should be kept in a dark bottle in the refrigerator.

Direct Prompt Reaction. Addition of 1 cc. of the reagent to 1 cc. of serum is followed by the immediate (in 20–30 seconds) appearance of a violet color.

Direct Delayed Reaction. A red color appears 1 minute after the reagent is added and gradually changes to violet.

Indirect Reaction. 2 cc. of 95 per cent alcohol are added to 1 cc. of serum, mixed, shaken, and centrifuged. A violet color promptly develops when 0.25 cc. of the reagent and 0.5 cc. of alcohol are added to 1 cc. of the supernatant fluid.

INTERPRETATION

Normal serum yields only a faint indirect reaction. The sole condition in which an intense indirect reaction is obtained is hemolytic jaundice, including icterus neonatorum. A prompt direct reaction develops in obstructive jaundice, whereas in parenchymatous jaundice the reaction is a delayed direct one. Table 9 (page 37) lists the results of qualitative tests for bile pigments in the different types of jaundice.

All serums which give a direct reaction also give the indirect reaction.

QUANTITATIVE (INDIRECT) VAN DEN BERGH'S TEST

This modification of the original test was developed by Thannhauser and Andersen (10). Since any bilirubin, after extraction with alcohol, gives the color reaction with the diazo reagent, the total bilirubin in serum can be estimated from the intensity of the reaction in the indirect van den Bergh test. The color is compared by visual colorimetry with standard solutions of bilirubin (11), or cobalt sulfate standards (12,13). When standard solutions of bilirubin are used, the result is expressed as milligrams of bilirubin per hundred cubic centimeters; with cobalt solutions, the bilirubin concentration is frequently reported in van den Bergh units per hundred cubic centimeters, one unit being equivalent to 0.4 or 0.5 mg. of bilirubin, depending on the composition of the standard employed.

A more accurate method than the one just described is the determination of serum bilirubin (page 33); it is now in general use.

ICTERUS INDEX

Meulengracht (14) is the author of this method. The test is still considered to be accurate and reliable (15) despite development of newer methods for a quantitative analysis of bilirubin in serum. To establish the icterus index, the yellow color of the serum is visually compared with an arbitrary, standard potassium dichromate solution. Of the many modifications of the original procedure that have been suggested, two will be described here, a macromethod as modified by Newburger (16) and a micromethod as devised by Davis (17).

PROCEDURE

Macromethod (16). 1 cc. anhydrous acetone is measured into a 12 × 100 mm. tube, 0.5 cc. serum is added, and the two are mixed and centrifuged for 5 minutes. The supernatant fluid is then transferred to the same type of tube as used for the standards, and is compared with the standard tubes in a comparator. If the sample is darker than any of the standards, the test must be repeated with serum that has been diluted with a known amount of acetone.

To prepare the standards, make 10 different dilutions in 10 test tubes of a stock solution containing 0.2 mg. potassium dichromate in 200 cc. of water. The tubes should contain from 1 to 10 cc. of stock solution and water up to 10 cc., plus 1 drop of concentrated hydrochloric acid. Transfer 2 cc. of each dilution to tubes of similar non-sol glass and of equal bore, seal, and number the tubes from 1 to 10. If kept in a dark place, the color will remain unchanged for many months.

The number of the standard dilution which matches the color of the sample, multiplied by the dilution of the serum, represents the icterus index.

Example: If 2 parts of acetone were added to 1 part of serum and the supernatant fluid matches standard dilution of tube 4, the icterus index is 12 (4 × 3 = 12).

Micromethod (17). *Apparatus.* Special glass capillary tubes. From glass tubing of uniform 2 mm. bore cut 10 cm. long tubes and draw out both ends of each tube to fine capillaries. The tubes must be clean and dry.

TABLE 7A
Standard Dilutions of Potassium Dichromate for Icterus Index by the Micromethod (17)

Cubic centimeters*	Resulting dilution	Icterus index
1	1:10,000	1
2	2:10,000	2
4	4:10,000	4
6	6:10,000	6
10	10:10,000	10
15	15:10,000	15
20	20:10,000	20
30	30:10,000	30
50	50:10,000	50
75	75:10,000	75
Undiluted	100:10,000	100

* Of 1:100 solution diluted to volume in a 100 cc. flask.

A 2 cm. column of blood from the finger tip of a fasting child is taken up in one of the capillary tubes. The blood is allowed to clot. The opposite, clear end of the tube is then sealed by a Bunsen flame, the tube is placed, sealed end down, in a padded centrifuge tube, and the clot thrown down by centrifugation. The layer of clear serum which forms above the clot is matched with standards contained in tubes of the same bore, and read by daylight. Hemolytic serums must be discarded.

To prepare the standards, place 1 Gm. of potassium dichromate in a 100 cc. volumetric flask and add water to volume; this gives a 1:100 dilution, or the standard solution of index 100. From this solution the 10 standard dilutions are prepared as shown in Table 7A.

The 10 standard dilutions may be introduced into the capillary tubes described above by means of a fine capillary pipet. Fill the tubes halfway, and seal the open ends. If kept in the dark, the standards remain good indefinitely.

INTERPRETATION

Except in early infancy, the normal icterus index during childhood is within the limits of 2 to 8. Values of 2 to 5 are more common than in adults (18). Values between 8 and 16 indicate a preicteric state, or latent jaundice; 16 is usually regarded as the critical value, visible jaundice being present when the index is above 16. The icterus index may rise to 35 or higher in grave forms of icterus, such as occurs in erythroblastosis foetalis or congenital atresia of the bile ducts.

The majority of newborn infants show an abnormally high icterus index, as a result of a physiologic hyperbilirubinemia. The peak is reached between the second and fourth days of life, after which the index gradually declines. An average normal figure is reached by the end of the second week, or at the very latest, by the third or fourth week. For a discussion of bilirubinemia in infants, see page 35.

DETERMINATION OF BILIRUBIN IN SERUM

The method described is that of Malloy and Evelyn (19); it is based on the same principle as the quantitative van den Bergh test. Their test, however, makes use of electrophotometry instead of visual colorimetry, thus eliminating turbidity of the serum as an interfering factor.

PROCEDURE

Apparatus. Photoelectrocolorimeter.

Reagents.

(*1*) Diazo reagent (page 30).

(*2*) Diazo blank. Dilute 15 cc. concentrated hydrochloric acid with water to 1 liter.

(*3*) Absolute methyl alcohol.

(*4*) Stock bilirubin solution. Dissolve 10 mg. bilirubin (available commercially) in reagent chloroform and dilute to 100 cc. with chloroform.

(*5*) Standard bilirubin solution. Transfer 10 cc. of stock solution to a 100 cc. volumetric flask and make up to volume with 95 per cent ethyl alcohol. 1 cc. is equivalent to 0.01 mg. bilirubin.

Technic. In a test tube 0.5 cc. serum is diluted with 9.5 cc. distilled water. In the order given, the following solutions are transferred into a colorimeter tube: 6 cc. methyl alcohol, 1 cc. freshly prepared diazo reagent, 5 cc. diluted serum. Hemolysis of the serum should be avoided, if possible; it does not, however, prevent fairly accurate results. Into a colorimeter tube labeled "blank" are transferred 6 cc. methyl alcohol, 1 cc. diazo blank, and 5 cc. diluted serum. After inverting each tube several times to mix the contents, the tubes are allowed to stand at room temperature for 30 minutes and then are read in the colorimeter. If bubbles form, they must be dislodged by tapping the tube gently on the table.

Filter No. 540 should be used for readings with the Evelyn colorimeter. The blank is placed in the instrument and the galvanometer set to read 100. The sample tube is then inserted and the galvanometer reading recorded. If the reading is below 10, readings are repeated after addition of 12 cc. of 50 per cent methyl alcohol to each tube. The results thus obtained are multiplied by 2.

Calculation. Bilirubin concentration in analyzed serum, expressed in milligrams per hundred cubic centimeters, is read directly from a calibration curve obtained by analysis of standard bilirubin dilutions. For this, 1, 2, 4, 6, and 8 cc. of the standard bilirubin solution are transferred to a series of tubes, each containing 1 cc. of diazo reagent, and sufficient ethyl alcohol is added to each tube to make a final volume of 10 cc. A blank tube is prepared by diluting 1 cc. of the diazo blank with 9 cc. of ethyl alcohol. The bilirubin concentrations in these standard tubes equal, respectively, 2, 5, 10, 15, and 20 mg. of bilirubin per hundred cubic centimeters of serum.

Another way of calculating bilirubin concentration is with the formula:

$$\text{mg. bilirubin per 100 cc. serum} = 100\ (L/K)$$

where $L = 2 - \log G$ (G = galvanometer reading), and K is the calibration constant obtained with standard solutions of bilirubin prepared as described above. As determined by the authors of the method, using the Evelyn colorimeter, the value of K is 3.5.

The calibration curves and constants may be used interchangeably. For further details with regard to obtaining the calibration curves and calibration constant, see page 206 and the directions supplied with the apparatus (20).

INTERPRETATION

It is generally accepted that the average normal level of bilirubin in serum in children and in adults is close to 0.5 mg. per hundred cubic centimeters, with a range of 0.25 to 0.75 mg. (21). Values which fall between the upper normal limit and 2 mg. per hundred cubic centimeters are the zone of latent jaundice; concentrations over 2 mg. as a rule are associated with clinical jaundice. The degree of hyperbilirubinemia is roughly parallel to the intensity of the concomitant jaundice.

TABLE 8

Maximum Bilirubin Levels in Serums of 106 Newborn Infants Selected at Random, Regardless of Day on Which Maximum Was Reached

Approximate percentage of infants examined	Bilirubin in serum, mg. per 100 cc.
19.6	under 2.0
23.6	2.5– 5.0
18.9	5.0– 7.5
12.5	7.5–10.0
14.1	10.0–12.5
7.1	12.5–15.0
3.1	15.0–17.5
0.7	17.0–20.0
0.7	over 20.0

After Davidson, Merritt, and Weech (22).

This interpretation of abnormally high bilirubin levels, however, cannot be applied to the newborn, the majority of whom develop a physiologic hyperbilirubinemia, with or without concomitant jaundice. From Table 8, which gives the normal quantitative changes in the serum bilirubin level during the neonatal period, it may be seen that the hyperbilirubinemia attains its highest degree between the second and fifth days of life. Figure 3 illustrates the frequency distribution of physiologic hyperbilirubinemia and its relation to clinical jaundice in the newborn. The chart reveals that: (*1*) There is no critical serum bilirubin level as regards visible jaundice. (*2*) Infants do not easily develop clinical jaundice during the neonatal period, the majority exhibiting no icterus until the concentration of bilirubin in serum is approximately 4 to 5 mg. per hundred cubic centimeters (23) (the corresponding value in van den Bergh units is 10.1). (*3*) There is no consistent correlation between the appearance and degree of jaundice and the intensity of hyperbilirubinemia (24).

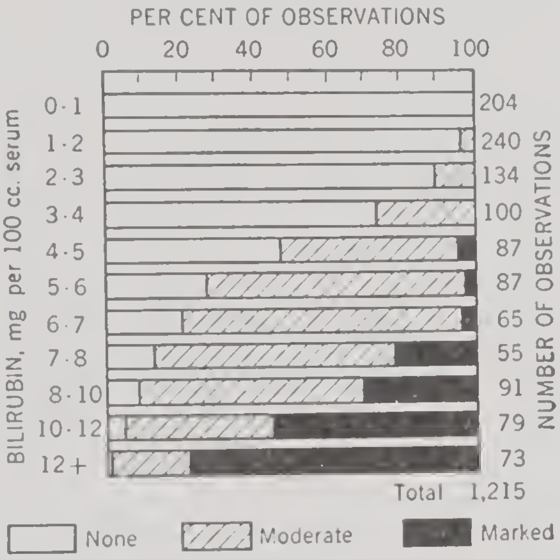

Fig. 3. Relation between bilirubin concentration in serum and degree of jaundice in young infants. From Davidson, Merritt, and Weech (22).

URINALYSIS FOR BILIRUBIN AND UROBILINOGEN

PROCEDURE

For Bilirubin. 2 cc. freshly voided urine are overlayed with a solution of 1 part tincture of iodine and 9 parts alcohol. If a green ring forms at the area of contact, the results of the test are positive.

Another reliable procedure is the Harrison test (25), in which

Fonchet's reagent (25 Gm. trichloroacetic acid, 100 cc. distilled water, and 10 cc. of 10 per cent ferric chloride) is used. To 10 cc. of urine are added 5–10 cc. of 10 per cent barium chloride solution, the precipitate is filtered off, and Fonchet's reagent is dropped on this precipitate. If the urine contained bilirubin, the characteristic blue-green color of biliverdin appears in the precipitate.

For Urobilinogen. 1 cc. of Ehrlich's aldehyde reagent is added to 10 cc. of fresh urine. To prepare the reagent, dissolve 2 Gm. *p*-dimethylaminobenzaldehyde in 100 cc. of 20 per cent hydrochloric acid. The appearance of a pink to cherry-red color after 5 minutes at room temperature is a positive reaction.

For a rough estimate of the urobilinogen concentration, the test is repeated with various dilutions of urine (1:10, 1:20, etc.) until a negative reaction is obtained. The result is expressed in terms of the dilution which still shows a pink color (25a).

INTERPRETATION

Bilirubin is normally not present in the urine. The incipient and recovery stages of parenchymatous jaundice are characterized by outspoken bilirubinuria and urobilinogenuria, while the advanced stage is marked by large amounts of bilirubin but only small amounts of urobilinogen in the urine.

TABLE 9
Results of Qualitative Tests for Bile Pigments in Jaundice

	Results				
		Jaundice			
Test	Normal	Obstructive	Parenchymatous	Hemolytic	Icterus neonatorum
Bilirubin in urine	0	+++	++	0	0*
Urobilinogen in urine	±	0	+	+++	+
Indirect van den Bergh	±	+++	++	+++	++
Direct (prompt) van den Bergh	0	+++	0	0	0
Direct (delayed) van den Bergh	0	0	++	0	0

* Very small amounts can be demonstrated by using sensitive methods.

In uncomplicated obstructive jaundice there is a pronounced urinary excretion of bilirubin, but almost no urobilinogen. When the obstruction is only partial, bilirubin and varying amounts of urobilinogen are found.

In hemolytic jaundice the urine contains large quantities of urobilinogen but no bilirubin (Table 9). A positive urobilinogen reaction obtained by a dilution not weaker than 1:20 is considered still normal (25a).

BROMSULFALEIN TEST

Rosenthal and White's method (26) makes use of a dye in an excretory loading test. Bromsulfalein (disodium phenoltetrabromophthalein sulfonate) is injected intravenously and the rate at which it disappears from the blood is measured. The rapidity with which its blood concentration decreases is a measure of the liver's excretory capacity. In the presence of icterus the test is not reliable, patients with obstructive or parenchymatous jaundice showing marked dye retention whatever the degree of hepatic insufficiency.

PEDIATRIC CONSIDERATIONS

Studies in children have not borne out the general assumption that in hepatogenic jaundice the retention of bilirubin (which accounts for the icterus) and the retention of bromsulfalein are parallel and caused by one and the same functional mechanism. A divergence between bromsulfalein excretion and bilirubin excretion has been observed, particularly in young children (27). To explain this finding, it has been suggested that in children the liver cells lack the functional maturity necessary to excrete "foreign" dye substances with the same ease as "physiologic" bile pigment. Furthermore, it has been asserted (28) that the bromsulfalein test does not probe the liver function but rather the function of the reticulo-endothelial system, including Kupffer's cells. The normal rate of excretion of bromsulfalein, for example, is attained by healthy infants only when they have reached the age of 4 to 5 months, which is long after the bilirubin in the blood has dropped from its high level at birth to normal. It is also known (29) that the results of the bromsulfalein test in cases of familial hemolytic jaundice are usually normal, despite the incapacity of the liver cells to prevent bilirubin retention, a fact which lends support to the general opinion that the reticulo-endothelial system functions normally in this disease. Such findings strengthen the conclusion that in young children a positive bromsulfalein test is not necessarily a sign of impaired liver function, and vice versa.

The foregoing is occasioned by the apparently growing tendency to use the test in children. Apart from the fact that the bromsulfalein test is not reliable in jaundiced children, pediatricians should be aware of its theoretic limitations.

The "serial" bromsulfalein test (30) will not be described here, because the repeated withdrawal of blood samples which it requires is difficult in small children.

PROCEDURE

The dose of bromsulfalein is 2 mg. per kilogram of body weight whatever the age of the child. A sterile, 5 per cent solution of the dye is commercially available, 1 cc. of which contains 50 mg. of dye; dosage with this solution is 0.04 cc. per kilogram of body weight. With the child in the fasting state, the total dose is injected intravenously within 60 seconds. Care should be taken that the dye does not escape into the tissues. After 30 minutes, 4–5 cc. of blood are withdrawn by venipuncture, using a different needle and syringe. The blood is transferred into a centrifuge tube and 1 drop of 20 per cent potassium oxalate is added. After centrifugation, the plasma is removed and compared with a series of standard dye solutions; the solution matching the color of the plasma is noted. If a sufficient amount of serum is obtainable, it can be used in like manner.

TABLE 10
Preparation of Standards for Bromsulfalein Test (26)

Strength of standard, per cent	Prepared by addition of alkaline water, cc.*	Prepared by addition to 100% standard, cc.†
10	4.5	0.5
20	4.0	1.0
30	3.5	1.5
40	3.0	2.0
50	2.5	2.5
60	2.0	3.0
70	1.5	3.5
80	1.0	4.0
90	0.5	4.5
100	0.0	5.0

* 0.25 cc. of 10 per cent sodium hydroxide in 100 cc. water.
† For composition, see page 40.

Standards. Transfer 0.08 cc. of the 5 per cent stock solution of bromsulfalein and 0.25 cc. of 10 per cent sodium hydroxide into a 100 cc. volumetric flask, and dilute with water to volume. This is the 100 per cent standard. In a set of small tubes prepare a series of dilutions at 10 per cent intervals, as shown in Table 10, and seal the tubes. If stored in the dark, the solutions keep for several months. Standards are also obtainable commercially.

Determinations. Equal amounts of plasma or serum (0.5–1 cc.) are transferred to each of a pair of tubes, of the same type as used for the standards. To one tube (control) 1 drop of 5 per cent hydrochloric acid is added to clarify the plasma. To the other tube (unknown) 1 drop of 10 per cent sodium hydroxide is added to develop the color of the dye. Visual comparison is done with a colorimeter of the Walpole type by placing the control tube in front of the standard, and a tube containing water behind the unknown. The standard solution which best matches the color of the unknown is taken as the reading.

INTERPRETATION

The plasma of normal children 1 year old and over shows only traces of the dye if the blood is withdrawn 30 minutes after injection of the test dose. Concentrations that are over 10 per cent are definitely abnormal. Infants retain the dye somewhat longer. Table 10A gives the average plasma concentration of bromsulfalein in healthy infants, measured 30 minutes after injection of the dye. Higher concentrations than those listed in the table must be considered abnormal and indicative of impaired liver function.

TABLE 10A

Average Concentration of Bromsulfalein in Plasma of Normal Infants 30 Minutes after Injection of Test Dose

Age	Concentration, per cent
Less than 24 hours	20–30
1.5–7 days	10–30
2–3 weeks	20–30
1–4 months	10
5–8 months	<10

After Herlitz (27)

BILIRUBIN EXCRETION TEST (PLASMA BILIRUBIN CLEARANCE TEST)

The desirability of using a physiologic material rather than a dye has led Eilbott (31) to recommend the bilirubin excretion test for hepatic function. The test determines the percentage of bilirubin, intravenously injected, that is present in the circulation 4 hours after its administration. In normal adults, no bilirubin is found at the end of this period; a retention of more than 5 per cent is considered evidence of liver damage. Various modifications of the test have been recommended (32). The test is believed to be one of the most sensitive methods for detecting impaired hepatic function in adults.

The test was so little used in children (33) that it was almost forgotten. But recently Weech and co-workers (34) have reported studies on children with a new quantitative method which estimates clearance of bilirubin from plasma and permits quantitative measurement of liver function. Their method does not depend upon the basal concentration of bilirubin in the plasma.

The special equipment required for the relatively complicated procedure of the bilirubin clearance test limits its usefulness in routine clinical work, despite the test's apparent superiority over the conventional excretion test. But the new method developed by Weech *et al.* may prove very useful in detailed pediatric investigations, including cases of jaundice which give an indirect van den Bergh reaction.

SERUM CHOLESTEROL PARTITION

The test is based on the principle that cholesterol occurs in the plasma in two forms—as free cholesterol and its ester, i.e., combined with fatty acids. It is very probable that synthesis of the esters and hydrolysis into their component parts is a function of the liver (35). In healthy individuals the ratio of free to combined cholesterol remains surprisingly constant, without regard to their actual concentrations. The chief abnormality in the partition of plasma cholesterol, as observed by Thannhauser (35), is a decrease of the ester fraction, resulting in an increased ratio of free to combined cholesterol above the normal narrow limits. This relative decrease in cholesterol esters has been generally recognized as an indication of diffuse liver damage.

Unlike most of the other constituents of plasma lipids, the level of total cholesterol in serum definitely varies with age. These variations must be considered when determinations of total plasma cholesterol in young children are used as a measure of total lipid concentration (page 134). The ratio of free to ester cholesterol shows a similar relation to age (Table 11). During the newborn period and for a short time thereafter healthy infants show a relatively low ester fraction that leads to increased ratios (36,37); in children over 4 weeks of age the ratio is almost identical with and as constant as that observed in adults. Aside from these physiologic variations, an abnormal ratio is as significant in children as it is in adults.

Two of the three fractions of cholesterol are determined by analysis, the third is computed. Micromethods have simplified the chemical analysis of the two fractions in children. Bloor's method (38), the one commonly used, requires 1.5 cc. of plasma for the fractional analysis, while the photoelectric determination devised by Schoenheimer and Sperry (39) uses only 0.2 cc. of serum or plasma. This method is described in detail on page 137.

TABLE 11

Cholesterol Partition in Plasma; Normal and in Diffuse Hepatic Damage

	Percentages		Ratios			
Age	Ester in total (37)	Free in total (36)	Free/ ester	Ester/ free (37)	Free/ total (40a)	Ester/ total
Normal						
Newborn	41–72	28–59*	0.4–1.4*	0.7–2.6	>0.32*	<0.7*
Over 4 weeks	↓	↓	↓	↓	↓	↓
Children and adults	70–75	24–30	0.3–0.4*	2.3–3.1	0.2–0.3	0.7–0.8*
Diffuse hepatic damage (any age)	<69*	>31	>0.5*	<2.0*	>0.32	<0.68*

* Computed by H. Behrendt.

Figures in parentheses are reference numbers.

Except in infants up to 1 month of age, reduced ester fractions are the result of liver disease. The abnormal relation of the cholesterol fractions which results from an absolute or relative depression of the ester fraction, is most clearly recognized by the ratio of free to ester cholesterol, or the percentage of ester cholesterol in total cholesterol. Other ratios have also been recommended (Table 11).

An increased ratio or a decreased percentage points to the presence of hepatic insufficiency. The partition of plasma cholesterol in some typical forms of liver disease is illustrated in Figure 4, while Table 11 gives the actual ratios and percentages pathognomonic of impaired liver function. The ratios seem to be a poor index of hepatic function in nonobstructive cirrhosis of the Laënnec type, as observed in children between the ages of 8 months and 10 years (40b). As Stoesser (41) was able to demonstrate, extensive infections of the upper respiratory tract, for example, pneumonia, are a common cause of liver injury and a marked fall in the ester fraction.

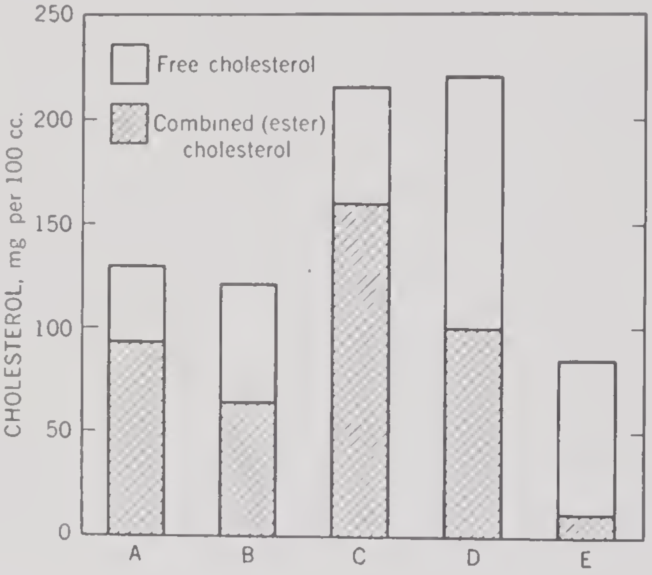

Fig. 4. Scheme showing cholesterol partition in plasma, according to Thannhauser and Schaber (35). A: Normal. B: Hepatic damage (hepatitis). C: Hepatic obstruction. D: Hepatic obstruction plus secondary hepatitis. E: Severe catarrhal jaundice.

CEPHALIN–CHOLESTEROL FLOCCULATION TEST

The test, devised by Hanger (42), represents the oldest of a number of flocculation tests for liver function. They are based on the principle that hepatocellular damage changes the normal pattern of serum proteins, particularly globulins, and that these changes can be demonstrated by the precipitation by such pathologic serums of certain test mediums. In the Hanger test, an emulsion of cephalin and cholesterol is used as substrate; serums from patients with hepatic damage will flocculate the emulsion. A positive reaction points to an impairment of that particular function of the liver which guarantees formation of normally composed serum globulins. The degree of a positive flocculation reaction reflects the state of the hepatic disease, rather than the potency of the residual liver function.

The technic and interpretation of the test in children is the same as in adults. The test requires only 0.2 cc. of serum.

PROCEDURE

From 2 to 4 cc. of blood are withdrawn and serum is obtained in the usual way. 1 cc. of the cephalin–cholesterol emulsion (available commercially) is added to a centrifuge tube containing 0.2 cc. of serum diluted with 4 cc. of 0.85 per cent saline solution. The tube is thoroughly shaken, stoppered with cotton, and then allowed to stand undisturbed at room temperature. The amount of flocculation and precipitation is recorded after 24 and after 48 hours.

INTERPRETATION

If the emulsion remains a stable, homogeneous suspension, the reaction is negative. When the reaction is positive, the lipoid material tends to flocculate and precipitate to the bottom of the tube. Complete precipitation, leaving a clear supernatant liquid, is designated as 4 plus; gradations are designed as 1 plus, 2 plus, and 3 plus.

TAKATA-ARA TEST

One of the physiologic functions of the liver is to secure the normal constitution of plasma globulins. When the liver is damaged, the physicochemical properties of serum globulin become abnormal. In the Takata-Ara test (43) these changes are demonstrated by the altered reaction of an alkaline sublimate solution to serum. Diluted serum of patients with hepatic damage will precipitate the solution, normal serum in corresponding dilutions will not. This test is generally considered as one of the more sensitive methods of detecting hepatocellular injury.

The technic and interpretation of the test are the same in children as in adults. The test requires 1 cc. of serum, i.e., 4 cc. of blood.

PROCEDURE

In the test, as modified by Ucko (44), 0.2 cc. of serum is transferred into each of 5 clean test tubes of 11 mm. diameter, and 0.1, 0.15, 0.2, 0.25, 0.3 cc. of 0.36 per cent anhydrous sodium carbonate are measured into the 5 tubes. The tubes are shaken, the same quantities of 0.5 per cent mercuric chloride solution are added, and the tubes are then shaken again. Readings are taken at once and after 90 minutes; the latter is decisive.

INTERPRETATION

The reaction is considered negative if the mixture in 3 or more tubes is translucent; the mixture may be clear or slightly opaque.

If the mixture in the first 3 tubes is turbid and not translucent, the reaction is considered to be positive and is graded 1 plus. If the mixtures in all 5 tubes show uniform turbidity and are not translucent, the reaction is graded as 2 plus. A reaction in which a heavy precipitate forms in all tubes immediately after the mercuric chloride is added is graded as 3 plus.

The diagnostic value of the Takata-Ara reaction in liver disease, and in hepatic cirrhosis in particular, has been the subject of many reports. For references, see Golob and Nussbaum (45), and Kirk (46), respectively.

COLLOIDAL GOLD REACTION IN SERUM

Like the Takata-Ara reaction and the cephalin–cholesterol flocculation test, Gray's gold-sol test on serum (47) shows the changes in plasma globulin which occur in hepatic insufficiency. It is based on the fact that colloidal gold chloride characteristically changes color when dilutions of serum containing pathologic globulins are added. The method is patterned on Lange's gold-sol test on spinal fluid.

The reaction has been accepted as a useful test of hepatic function in adults. In children, however, its value has not yet been established by large-scale studies. The method will nevertheless be described here because gold-sol reactions are now frequently included in the case histories of children.

PROCEDURE

According to Gray's original directions, enough venous blood is drawn from the fasting patient to obtain 0.2 cc. of serum. After separating the serum from the clot, by centrifugation if necessary, 0.1 cc. of the serum is diluted 1:350 with physiologic sodium chloride solution. 1.8 cc. of 0.3 per cent solution of sodium chloride are measured into the first of a series of 10 tubes, and 1 cc. of the solution into each of the following 9 tubes. Then 0.2 cc. of the diluted serum is added to the first tube, the contents are mixed, and 1 cc. of the mixture is transferred to the second tube and mixed. This procedure is followed through the whole series of tubes. After discarding 1 cc. of the mixture from the last tube, 5 cc. of colloidal gold solution of standardized acidity (available commercially) are added to each tube. The tubes are read 12 to 24 hours later, and readings are recorded as "indices" by the system used for Lange's reaction in spinal fluid as follows:

Index	Color of fluid	Precipitation
5	None	Complete
4	Light blue	Intermediate
3	Blue	Intermediate
2	Orchid	Intermediate
1	Red-blue	Intermediate
0	Red	None

The readings of all the tubes, in the order of increasing dilutions, represent a row of 10 numbers, and are called the gold-sol curve.

Andersch (48) recommends a *micromodification,* using smaller quantities of serum. Blood from the finger tip is drawn in a glass capillary tube of 2 mm. bore, the opposite end is sealed in a flame, and the tube is centrifuged. The tube is then broken at the line of demarcation between cells and serum, and the serum is drawn into a 20 c. mm. hemoglobin pipet and washed into 7 cc. of physiologic solution of sodium chloride. This gives the initial dilution of 1:350. The test is then continued according to Gray's original method.

Another modification has been introduced by Maclagan (49), who recommends the use of a barbital buffer instead of sodium chloride for diluting the serum. As the reaction (*p*H) is controlled by the buffer solution, the gold-sol need not be standardized, thus simplifying its preparation.

INTERPRETATION

The normal range of the reaction is from 0000000000 to 3332200000; and all numbers falling within this range are considered as negative results of the test. Only occasionally does the number 3 appear on the left side of the normal curve.

A positive reaction is characterized by numbers above 3 on the left side of the curve, for example, 55532100000 or 5432100000.

This curve resembles the dementia paralytica type of curve obtained with spinal fluid. Precipitation beyond the sixth tube to the right is exceptional.

The results of the test are almost uniformly negative in obstructive jaundice, but positive in infectious hepatitis and cirrhosis.

THYMOL TURBIDITY TEST

This test has been developed by Maclagan (50a), who observed that a barbital buffer saturated with thymol becomes turbid upon addition of serum from patients with hepatic damage. The reaction is probably caused by some globulin fraction which is released into the blood stream in unusually great amounts when there is parenchymal liver damage. As compared to the cephalin–cholesterol flocculation and colloidal gold tests, the thymol turbidity test cannot be considered as more sensitive, even when the thymol reaction only is positive in a given condition. Either test provides information about a different fraction of serum globulin and a different type of hepatic involvement. In combination, these tests are of greater diagnostic value than either one alone (50b). Cephalin–cholesterol flocculation, for instance, is likely to be related to high gamma-globulin levels, whereas thymol turbidity is probably produced by the beta-globulin fraction (50c). Thus, it is the type of liver disease and not the relative sensitivity of the various tests which is responsible for the discrepancy or uniformity of their results in a given patient.

PROCEDURE

Reagent. Place 1.03 Gm. sodium barbital, 1.38 Gm. barbital, and 3 Gm. powdered thymol crystals in a 1,000 cc. Erlenmeyer flask, add 500 cc. distilled water, and heat the solution to the boiling point. Remove flask from the flame, mix contents by shaking, and cool to room temperature. The solution becomes turbid as it cools. Add a small quantity of powdered thymol crystals to the cooled solution, mix by shaking, stopper the flask, and leave at room temperature overnight. Thymol crystals form at the bottom of the flask; mix by shaking and filter. The clear filtrate, which keeps indefinitely at room temperature, is used as the reagent.

To 3 cc. of the reagent is added 0.05 cc. serum in a 10 × 75 mm. cuvet, and the two thoroughly mixed. After 30 minutes the degree of turbidity is determined by Maclagan's method or by Shank and Hoagland's modification (51).

Maclagan's Method. Turbidity is measured by visual comparison in a comparator with a set of standards (commercially available) devised by Kingsbury and co-workers (52a) for estimating albumin in urine and spinal fluid. A detailed description of the preparation of the standards will be found on page 488. If the turbidity of the specimen being tested exceeds the 100 mg. standard, it must be further diluted with a measured volume of reagent.

The standards are suspensions of formazin in gelatin standardized at various degrees of turbidity; they are graded to correspond with albumin concentrations ranging from 10 to 100 mg. per hundred cubic centimeters in 10 standard tubes.

The results are expressed in arbitrary units, which equal the appropriate standard divided by 10, with allowance for the dilution of the serum. With the standard procedure, the dilution of the serum is 1:60.

Calculation.

$$\text{Units turbidity in serum} = \frac{\text{standard tube reading} \times \text{final dilution of serum}}{10 \times 60}$$

Example. If the final dilution of serum is 120 and the mixture matches the 70 mg. per cent standard tube, the thymol turbidity of the serum is 14 units.

Shank and Hoagland's Modification (51a). Turbidity is measured in a spectrophotometer at a wave length of 650 $m\mu$. The galvanometer is adjusted to 100 per cent transmission with a blank containing 3.0 cc. of the thymol–barbital reagent. The cuvets should be well shaken just before readings are made. The turbidity of a given reaction is expressed in units derived from a standard curve prepared by using standard barium sulfate suspensions, plotting optical density against concentration.

To prepare these standards, 3.0 cc. of 0.0962 M barium chloride solution are diluted to volume in a 100 cc. volumetric flask by adding 0.2 N sulfuric acid at 10 C. This gives a relatively stable suspension of barium sulfate. For a 10 unit turbidity standard, add 1.65 cc. of 0.2 N sulfuric acid to 1.35 cc. of the barium sulfate suspension in a 10 × 75 mm. cuvet. For a 20 unit standard, add 0.3 cc. of 0.2 N sulfuric acid to 2.7 cc. of the barium sulfate suspension. In this way approximately 6 standards are prepared containing from 0.21 to 3.0 cc. barium sulfate suspension, corresponding to 1.5–22 units turbidity. At a wave length of 650 mμ, there is a straight line relationship between the optical density of the various dilutions of the barium sulfate standard, with distilled water as a blank.

The Evans blue (T-1824) standard, recommended by Kunkel and Hoagland (52b), appears to have certain advantages over the barium sulfate standard. In preparing and using these dye standards, a concentration of 3 γ per cc. is considered equivalent to 20 thymol turbidity units.

INTERPRETATION

With either of these two technics, normal readings are between zero and 4 units, with a mean average of 2.66 units. Serums from patients with parenchymatous liver disease show increased turbidity, the degree varying with the extent of damage. In parenchymatous jaundice, for example, the turbidity values average 17 units. In hepatitis, "the thymol test usually continues positive for a longer period of time during convalescence and correlates better with the disappearance of symptoms than the cephalin–cholesterol flocculation test" (52c).

Thymol Flocculation Test. According to Neefe (52c), this test "merely represents a reading of the degree of flocculation occurring after approximately 18 hours in the thymol–serum mixtures used for the 30 minute turbidity reading." The mixtures are allowed to stand at room temperature in subdued light. The results are read as in the cephalin–cholesterol flocculation test, the degree of floccluation being graded from zero to 4 plus. Grading over 1 plus is regarded as abnormal. As compared to turbidity readings, the flocculation test is of superior sensitivity.

PROTHROMBIN TIME DETERMINATION

According to Quick (53), the clotting process can be expressed as follows: (*1*) prothrombin + thromboplastin + calcium = thrombin, and (*2*) fibrinogen + thrombin = fibrin. Quick also recognized "that the clotting time of blood or plasma is a quantitative measure of the prothrombin concentration provided an excess of thromboplastin and a constant concentration of calcium are present." This is the basis of the prothrombin test, for obviously a prothrombin deficiency must lead to changes in the clotting properties of the blood. It is now generally accepted that vitamin K is involved in the formation of prothrombin (54), and that this synthesis is accomplished in the liver (55). Intestinal absorption of vitamin K and normal liver function are thus essential for maintenance of a normal prothrombin level in the blood (56). Hence, determination of prothrombin time reveals whether it is lack of prothrombin which has caused the change in blood clotting, but the test does not disclose the pathogenesis of prothrombin deficiency.

The test measures the length of time it takes to transform prothrombin into thrombin and to form the clot, the process being set in motion by adding thromboplastin and/or calcium. In this test the length of time is a measure of the actual amount of prothrombin present in the blood (prothrombin time).

PEDIATRIC CONSIDERATIONS

In early infancy there is a physiologic hypoprothrombinemia, with prolonged clotting time (56). There are several convenient simplifications of the original test method which may be used in children. Two of the most accurate—Quick's (58) and Kato's (60) are described below. A simple bedside test for rough quantitative estimations has also been recommended (57).

PROCEDURES

Quick's Simplified Test (58). The reagent in the test is thromboplastin, prepared as directed by Quick (53) from brain of freshly killed rabbits. Satisfactory potent preparations are also commercially available. Russell's viper venom (stypven), a thromboplastin-like substance, has also been used by some workers (59).

A drop of capillary blood, obtained by puncture, is put on a glass slide, and mixed with a drop of thromboplastin by slow stirring with a fine-pointed stirring rod. To note clotting the slide should be held over a light. The exact time interval between the addition of thromboplastin and the appearance of a clot, which represents the prothrombin time, is determined by stop watch.

Kato's Microtest (60). Hanging drop slides are prepared by coating the hollow of each with 0.02 cc. of a 2 per cent double oxalate solution and allowing the slides to dry at room temperature. The solution consists of 0.75 Gm. potassium oxalate, 1.25 Gm. ammonium oxalate, and distilled water to 100 cc. Approximately 0.2 cc. of capillary blood from a deep puncture in the heel, big toe, or finger tip is transferred into the hollow of a prepared hanging drop slide and at once thoroughly mixed with the dry oxalate by rotating the slide. The slide is then placed in a moist chamber (Petri dish with a moist filter paper) until the test is performed.

Exactly 0.1 cc. each of thromboplastin suspension (see above) and of $^1/_{40}$ M calcium chloride solution are measured into the well of a hanging drop slide and mixed. The solution consists of 1.11 Gm. anhydrous calcium chloride and 400 cc. distilled water. The mixing may also be done in a white porcelain spot plate; its 12 circular depressions permit convenient performance of several tests in rapid succession. 0.1 cc. of the oxalated blood from the slide preserved in the moist chamber is quickly added to the mixture and the stop watch is clicked simultaneously. The ingredients are mixed, and then agitated for 5–6 seconds with a fine glass rod. The stop watch is clicked again as soon as a gelatinous clot has formed —the end point of the reaction. The elapsed time is the prothrombin clotting time.

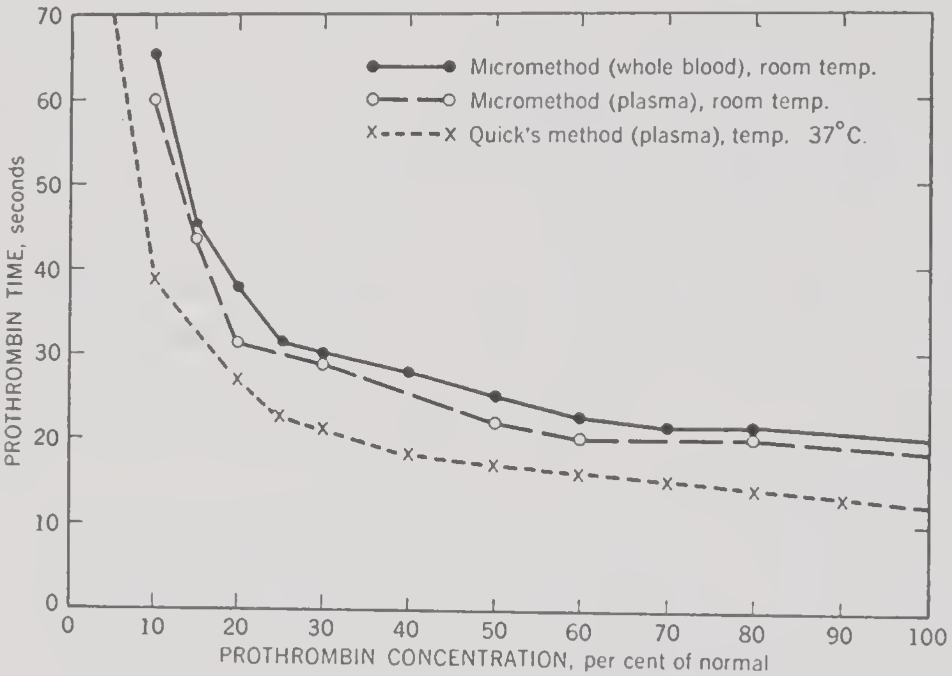

Fig. 5. Relation between prothrombin concentration in blood or plasma and prothrombin clotting time. From Kato and Poncher (56).

Reports on prothrombin tests should include both the method used and the type of blood (whole capillary, venous, or plasma).

Prothrombin clotting time is generally expressed in terms of seconds required for definite formation of a gelatinous clot. The result may also be expressed as a percentage of normal prothrombin concentration. This is shown in Figure 5, which also illustrates the relation of prothrombin clotting time to prothrombin concentration. Normal prothrombin time is considered equivalent to normal prothrombin concentration, i.e., 100 per cent concentration. Higher values of prothrombin time indicate decreasing prothrombin concentration.

INTERPRETATION

In adults and older children the average normal prothrombin clotting time, as determined with recalcified whole capillary blood, is about 15 to 20 seconds. In normal infants and young children, the average normal values are around 25 seconds, while in the newborn the normal average is still higher. Kato and Poncher (56) found an average prothrombin time of 43 seconds during the first day of life. As the infant grows older, there is a gradual decrease in the value until by the ninth or tenth day it reaches the young children's normal average of 25 seconds.

The range of abnormally high prothrombin time is from 30 to more than 300 seconds. They are found in various groups of childhood diseases, the most important being the hemorrhagic disorders of the newborn (melena neonatorum, hematemesis, hematuria, cerebral hemorrhage); in these disorders the physiologic vitamin K deficiency is an essential etiologic factor (61a). The celiac syndrome, severe diarrhea in infants (61b), and obstructive jaundice form another group; hypoprothrombinemia may develop in this group as a result of nondietary vitamin K deficiency. The fat-soluble vitamin is actually present in the intestines, but its absorption is impaired. When a lowered prothrombin level appears in diseases associated with acute or chronic hepatocellular damage, it is due to inadequate synthesis of prothrombin in the liver. Apart from the liver diseases proper, all sorts of poisoning may thus prolong the prothrombin time; salicylate intoxication, for example, is one poisoning which has recently attracted pediatric consideration (61c).

One fact that should be remembered in connection with blood clotting tests is that "clotting time" of blood shows practically no change until the prothrombin concentration has dropped to about

30 per cent of normal (Fig. 5). At that level, any of the methods which simply determine the "clotting time" (e.g., the Rodda test, page 477) will show delayed clotting. It is only the prothrombin clotting time test which discloses a decrease in prothrombin before the critical level of 30 per cent has been reached, and thus reveals the latent danger of hemorrhage.

RESPONSE OF PROTHROMBIN TIME TO VITAMIN K

Maintenance of normal prothrombin formation depends on (*1*) the functional integrity of the liver cells, and (*2*) the availability of sufficient amounts of vitamin K in the liver. If the vitamin deficiency has caused the prothrombin deficit, parenterally administered vitamin K will correct the situation; if injury to the functional capacity of the liver is the cause, prothrombin production will remain defective despite an increased supply of the vitamin. Hence, the response of an abnormal prothrombin level to administration of vitamin K discloses possible hepatocellular dysfunction as well as possible vitamin deficiency (62,63).

To test the response of the prothrombin level to vitamin K, the child with an abnormal prothrombin time, as previously established, is given a test dose (1 mg. intramuscularly or 2 mg. subcutaneously) of a synthetic vitamin K preparation (2-methyl-1,4-naphthoquinone, or 4-amino-2-methylnaphthol hydrochloride). About 24 hours later the prothrombin time is again determined, and the result compared with the first determination.

If the vitamin administration has restored the prothrombin clotting time to normal or almost normal, it may be assumed that the hepatic function is essentially unharmed. Such prompt response to vitamin K, in the absence of jaundice, is proof that avitaminosis K caused the prothrombin deficit; if jaundice is present, however, a diagnosis of obstructive icterus is indicated (63). Persistence of the prothrombin deficit, or a merely slight increase in prothrombin time after vitamin K administration is evidence of severe liver damage (64).

The average prothrombin time in hemorrhagic disease of the newborn is 209 seconds, or about 1.5 per cent of normal; after a day of vitamin K therapy, the average value is 26 seconds, or 80 per cent of normal (56). This proves that the prothrombin deficiency in these disorders is due to lack of vitamin K and is not the result of hepatic dysfunction (65).

If administration of vitamin K increases an originally normal prothrombin time, the presence of hepatic disturbances should be suspected, unless other reasons are revealed (66).

HIPPURIC ACID CONJUGATION TEST

The test, as developed by Quick (65), is based on the principle that when sodium benzoate reaches the liver via the circulation the benzoic acid radical is conjugated with glycine to form hippuric acid, and the acid is excreted in the urine. The liver's capacity to furnish the necessary amount of glycine and to synthesize the hippuric acid is determined by the amount of acid excreted after a standard dose of sodium benzoate. Individuals with hepatic insufficiency show a diminished urinary excretion of hippuric acid, as compared to normal excretion.

PEDIATRIC CONSIDERATIONS

So far as known, children and adults have the same capacity to form and excrete hippuric acid. However, children under 3 years old have not yet been tested systematically. The test doses suggested for children seem large, and are probably larger than necessary. This, however, does not affect the accuracy of the test, since the maximum amount of hippuric acid that can be formed per hour is practically fixed.

It has recently been suggested that the dose of benzoic acid be administered intravenously. As an alternate route when testing children, it offers definite advantages, only one quantitative collection of urine and a single urinalysis being required; furthermore, the child's cooperation in taking the drug is not essential and nausea and vomiting are prevented.

In adults and children alike, the test is considered highly significant of normal or impeded hepatic function (page 27). However, in the presence of dehydration, renal insufficiency, or obstruction of the urinary tract, the test is ineffective.

PROCEDURE

Oral Test. By Quick's method, as modified by Londe and Probstein (67), the child is given the test dose by mouth 1 hour after breakfast of tea and a piece of toast. The dosage is 3 Gm. of sodium benzoate for children weighing less than 40 Kg. and 4 Gm. for children over 40 Kg. (0.074–0.19 Gm. per kilogram of body weight), dissolved in 20 cc. of cold water. To make the solution palatable, saccharine, peppermint, and chocolate syrup may be added.

Urine is collected 2 hours and 4 hours after administration of the test dose. Each specimen is measured; if it is more than 125 cc. the urine is transferred to a beaker and placed on a steam bath, a few drops of glacial acetic acid are added, and the contents evaporated to about 75 cc. Analysis for hippuric acid should be carried out in each specimen separately.

After adding 30 Gm. of sodium chloride per 100 cc. of urine, the specimen is heated in a beaker, under constant shaking, until all the salt has dissolved. The beaker is then immediately immersed in ice-cold water and the contents are cooled to about 15 to 20 C., after which 1 to 2 cc. of concentrated hydrochloric acid are added and the contents vigorously stirred until precipitation of the hippuric acid is complete. The sides of the beaker must be scratched with a glass rod to enhance the crystallization. The beaker is replaced in cold water for 15 minutes; the contents are then filtered through a Hirsch funnel (diameter of perforated plate, 47 mm.), using moderate suction. The precipitate on the filter is washed with chilled 30 per cent sodium chloride from a wash bottle, using the washing fluid first to rinse the beaker in which the precipitation was performed. However, the precipitate need not be transferred quantitatively to the filter. Washing is continued until the filtrate is free of concentrated hydrochloric acid when tested by Congo paper. The funnel with the washed precipitate is then transferred into the beaker in which the precipitation was performed. The filtered hippuric acid is rinsed from the filter by hot water and thus dissolved. All of the hippuric acid is now in the beaker in which it was precipitated. The particles of hippuric acid adhering to the beaker are dissolved by gentle heating, and while still hot, the contents of the beaker are titrated with 0.5 *N* sodium hydroxide, using phenolphthalein as an indicator.

Calculation. By multiplying the number of cubic centimeters of 0.5 *N* sodium hydroxide used in the titration by 0.072, one obtains the grams of sodium benzoate excreted as hippuric acid in the analyzed specimen. By adding to this figure the amount of 0.1 for every 100 cc. of urine analyzed (or for every 30 Gm. of sodium chloride used), correction is made for that small amount of hippuric acid which is retained in solution and escapes determination. The final result is usually expressed as a percentage of the ingested dose of benzoate.

Example.

Ingested test dose = 4 Gm. sodium benzoate
Volume of first 2 hour urine specimen = 158 cc.
Result of titration = 22.5 cc. of 0.5 *N* sodium hydroxide
Corresponding value of sodium benzoate = 22.5 × 0.072 = 1.62 Gm.
Correction for solubility = 1.58 × 0.1 = 0.158 Gm.
Sodium benzoate content of specimen = 1.78 Gm.
Excretion in first 2 hours after ingestion = 44.5 per cent of test dose.

Intravenous Test. Quick's method (68) is to inject the test dose in the morning, with the child in a fasting state. The injection should be made very slowly, taking 5 to 10 minutes. The dose is 1.77 Gm. of sodium benzoate dissolved in 20 cc. of distilled water, and ampules are commercially available. Urine is collected shortly before the test and exactly 1 hour after the injection is completed. Only the second specimen is analyzed for hippuric acid.

The urine is measured and 5 Gm. of solid ammonium sulfate are added for every 10 cc. of urine. When the salt has dissolved, the urine is filtered and enough concentrated hydrochloric acid is added to acidify the urine distinctly, as indicated by Congo red paper. The solution is stirred until precipitation of the hippuric acid is complete; scratching the sides of the flask with a glass rod enhances crystallization. After 30 minutes, the crystalline product is filtered off quantitatively in a Buchner filter (diameter of filter paper, 4.5 cm.; of filter plate, 2.5 cm.). The filter paper must be weighed before use. The precipitate on the filter is washed with small quantities of cold water, and allowed to dry in air. The filter paper plus dried precipitate are weighed to the second decimal place. This weight, minus the weight of the empty filter paper, gives the weight of hippuric acid.

Calculation. To the weight obtained, add 0.12 Gm. for every 100 cc. of urine; this is the correction for the fraction of hippuric acid retained in solution. The total represents the amount of hippuric acid excreted in the urine within 1 hour after administration of the test dose.

INTERPRETATION

Oral Test. The limits of normal excretion have been established for children 3–15 years old (67), but no standards are available yet for children under 3. Table 12 gives the range of normal values and a definition of abnormal tests results. An abnormally low excretion is considered significant of impaired hepatic function.

TABLE 12

Results of Oral Hippuric Acid Test in Children Weighing 20 Kilograms and Over

Weight, Kg.	Test dose, sodium benzoate, Gm.	Per cent test dose excreted			
		Normal		Abnormal	
		in 2 hrs.	in 4 hrs.	in 2 hrs.	in 4 hrs.
20–40	3	32–58	67–88	<30	<65
Over 40	4	41–74	69–92	<40	<67

After Londe and Probstein (67).

Intravenous Test. According to Meneghello and Drinberg (69), normal children between 5 and 11 excrete an average of 1.1 Gm. of hippuric acid during the first hour after injection of the test dose. The lower limit of normal is considered to be 0.9 Gm. Values as low as 0.39 Gm. have been observed in abnormal reactions to the test dose. Abnormally reduced excretion is found in all clinical conditions causing impaired liver function.

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CHAPTER III

General Metabolism Tests. Respiratory Exchanges

DETERMINATION OF BASAL METABOLIC RATE

The basal metabolism is the sum of the metabolic processes which go on in the human body under the conditions of complete physical and mental repose, normal temperature, and a fasting state. These are known as basal conditions. "Basal metabolic rate" (B.M.R.) is the term applied to the amount of energy or heat which the basal metabolism produces. The basal metabolic rate is determined by measuring the oxygen consumption and computing the energy output from it (indirect calorimetry). The results are expressed in plus or minus per cent deviation from normals

The laws governing heat production under basal conditions, first recognized by Rubner (1), are the basis of our knowledge of the physiologic principles of basal metabolism and its measurement. Many have contributed to this knowledge in this field; some of the classic presentations of this subject are those of Krogh, Lusk, Benedict, Talbot, DuBois (2–6).

Among the accepted principles are the following: (*1*) Under basal conditions, the intensity of metabolic transformations in tissue cells tends to remain constant (7). (*2*) By means of formulas or tables the normal rate of heat production can be predicted from the body measurements (8). The classic data on basal metabolism have been reported in terms of Calories referred to body surface area (6,9), or to weight, height, and age (10). (*3*) Age is an important factor in the rate of basal heat production (Fig. 6), the relation between rate and body measurements remaining constant only in individuals of comparable age (4). (*4*)

Sex, diet, sleep, muscular tone, and pulse rate are other factors affecting basal metabolism (11).

To establish an individual's basal metabolic rate, his oxygen intake is measured by means of a respiration machine. Some apparatus measure both oxygen intake and carbon dioxide output, others measure only oxygen consumption; either type may be used. Heat production, expressed in Calories is then computed, by means of tables, from the oxygen consumption per unit of time, as revealed by the test. The values thus obtained are compared with normal standards, and the increase or decrease is expressed as plus or minus per cent (B.M.R.).

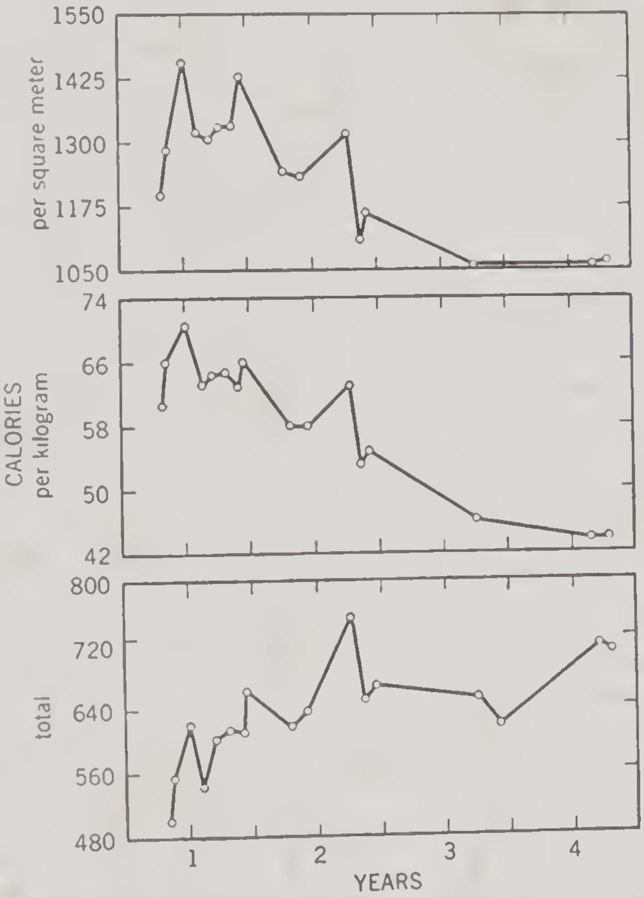


Fig. 6. Basal heat production per 24 hours in a normal child from the age of 10 months to 5 years. From Grafe (11), based on data reported by Benedict and Talbot (4).

PEDIATRIC CONSIDERATIONS

Determination of the basal metabolic rate in children is complicated by problems not encountered in adults. Attainment of satisfactory basal conditions involves technical difficulties; and choice of standards in interpreting the results of the test call for careful judgment.

Technical Difficulties. Any one of the portable or nonportable clinical respiration machines with a mouthpiece and flexible tubing may be used for children over 6 years of age. However, while some children readily adapt themselves to the technic of the breathing test, others can only with difficulty be induced to breathe regularly, to relax, not to be frightened or uneasy, and to lie quietly. Frequently tests must be discontinued because these basal conditions cannot be attained.

For the success and reliability of the basal metabolism test in children over 6, ability to handle children is of far greater importance than technical skill. A vast amount of patience and a good deal of imagination are called for. Sometimes it may be necessary to put the child through several fictitious test sessions so that the child becomes used to the necessary breathing procedure and the mouth valve and nose clip. Often, three determinations, instead of the usual two, must be made to obtain more reliable averages; and even then the results must be examined very critically. The results cannot be relied on if, during the test, the respiration is irregular in depth or the pulse rate is high because of nervous irritation. A difference of more than 5 per cent in results of tests performed on the same day, or on consecutive days, is a sign that basal conditions, respiratory technic, or both, were unsatisfactory; the test must then be repeated. For fairly reliable test results, careful attention must be paid to all these elements of error and potential misinterpretation.

For infants and children up to the age of 6 to 7 years, a respiratory chamber must be used. The child is placed in a closed compartment, air is circulated through it, and the oxygen consumed is determined. In the last decades these respiratory chambers have been greatly perfected and simplified. When Heubner and Rubner (12) in 1880 used the calorimeter for the first investigation ever made on the energy exchange in young children, 24 hour periods of air analysis and a score of attendants were needed. The modern test, as developed by Benedict and Talbot (13), requires less than half a day, the test periods lasting only 2 to 3 hours each. A later modification by Higgins and Bates (14) has so simplified the test that observation periods are only 2 to 4 minutes each, and the whole test can be performed in an hour.

Basal conditions for children tested in a respiratory chamber differ somewhat from those defined above. Infants are given their bottle just before the test starts; they then usually fall asleep and are completely relaxed. The error in metabolic rate as a result of the intake of food is not as serious as the one caused by crying and hunger. The most difficult problem is attainment of fair basal conditions for children 2 to 6 years old. If all other methods fail, the child may be fed as usual and the test performed at night, when the child is naturally sleepy (15). Sedatives should not be administered.

While respiratory chambers have provided the most important data on energy exchanges in young children, so far only a limited number of laboratories are using them.

Choice of Standards. Data on normal basal heat production of children from birth to adolescence are voluminous, but unfortunately these tables of standards are not uniform. This lack of uniformity is the result of investigations carried out in dissimilar groups of children, geographic, racial, economic, and dietary factors accounting for some of the differences. An additional factor is the use of different methods of calorimetry.

An apparent disagreement among investigators as to which body measurements relate most closely to basal metabolism in children introduces a further element of uncertainty. Some maintain that caloric measurements should be referred to body surface area, as commonly done in adults, others prefer reference to height, and still others refer basal metabolic rate to weight. But the standards even for each of these methods vary considerably, so that for a normal 8 year old boy, for example, various authors have found various "standard Calories for height." For practical purposes, one must choose one or another of the available standards. However, instead of choosing one procedure and ignoring all the others, a mode of computation will be discussed here which takes into account all methods of reference but so restricts their use as to reduce possible errors.

B.M.R. Computed with Standards Referring to Body Measurements. The first step is to determine whether the child's physical development is normal or abnormal, by comparing the child's weight and height with the respective normal averages. Body size is considered abnormal if height or weight is at least 20 per cent above or 10 per cent below the normal, as given in tables of standards (16).

Any one of the metabolic standards, whether they refer to weight, height, surface area, or square meter per age, may be used for children of normal physique, i.e., with weight and height falling within normal limits. Provided the child's body size is normal, the present routine practice of referring caloric measurements to surface area only is unobjectionable. The same tables for normal weight and height should be used as were employed by the author of the chosen metabolic standard.

TABLE 13

Percentage Deviation in Basal Metabolism of Three Subjects, in Terms of Calories per Hour Referred to Various Body Measurements

Measurements	Subject 1	Subject 2	Subject 3
Age	8 yrs. 9 mos.	8 yrs. 9 mos.	8 yrs. 8 mos.
Weight, Kg.	29.4	17.8	39.9
Height, cm.	135.9	112.9	141.2
Surface area, sq.m.	1.055	0.750	1.245
Percentage deviation from standards			
Cal./hr./sq. m./age	−7	+14	−1
Cal./hr./surf. area	−6	+1	+8
Cal./hr./wt.	−6	+2	+2
Cal./hr./ht.	−6	−2	+14

From Lewis, Duval, and Iliff (17).

However, greater discrimination in the choice of caloric standards is called for when body size is abnormal. Table 13 illustrates the disparate results which may be obtained when the various methods of reference are used on subjects of almost identical age but different physical development. In the first subject, whose body measurements are within the normal range, the results remain practically the same, whatever the standard of reference. But in the second and third subjects, who are of abnormal weight and height, the basal metabolic rate differs with different standards of reference.

According to Talbot (16), in children of abnormal body size

TABLE 14. Weight–Height–Age Table for Boys, from Birth to School Age. From Woodbury (22)

Ht., in.	Av. wt. for ht., lbs.	Age, months											
		1	3	6	9	12	18	24	30	36	48	60	72
20	8	8											
21	9½	9	10										
22	10½	10	11										
23	12	11	12	13									
24	13½	12	13	14									
25	15	13	14	15	16								
26	16½		15	17	17	18							
27	18		16	18	18	19							
28	19½			19	19	20	20						
29	20½			20	21	21	21						
30	22			22	22	22	22	22					
31	23				23	23	23	23	24				
32	24½				24	24	24	25	25				
33	26					26	26	26	26	26			
34	27						27	27	27	27			
35	29½						29	29	29	29	29		
36	31							30	31	31	31		
37	32							32	32	32	32	32	
38	33½								33	33	33	34	
39	35								35	35	35	35	
40	36½									36	36	36	36
41	38										38	38	38
42	39½										39	39	39
43	41½										41	41	41
44	43½											43	43
45	45½											45	45
46	48												48
47	50												50
48	52½												52
49	55												55

Weight is given to the nearest pound; height, to the nearest inch; age, to the nearest month. Up to and including 34 inches, weights are net; above this the following amounts have been added for clothing (shoes, coats, and sweaters not included): 35–39 inches, 1¼ pounds; 40–44 inches, 1½ pounds; 45–49 inches, 1¾ pounds.

TABLE 15. Weight–Height–Age Table for Girls, from Birth to School Age. From Woodbury (22)

Ht., in.	Av. wt. for ht., lbs.	Age, months											
		1	3	6	9	12	18	24	30	36	48	60	72
20	8	8											
21	9	9	10										
22	10½	10	11										
23	12	11	12	13									
24	13½	12	13	14	14								
25	15	13	14	15	15								
26	16½		15	16	17	17							
27	17½		16	17	18	18							
28	19			19	19	19	19						
29	20			19	20	20	20						
30	21½			21	21	21	21	21					
31	22½				22	22	23	23	23				
32	24					23	24	24	24	25			
33	25						25	25	25	26			
34	26½						26	26	26	27			
35	29						29	29	29	29	29		
36	30							30	30	30	30	31	
37	31½							31	31	31	31	32	
38	32½								33	33	33	33	
39	34								34	34	34	34	34
40	35½									35	36	36	36
41	37½										37	37	37
42	39										39	39	39
43	41										40	41	41
44	42½											42	42
45	45												45
46	47½												47
47	50												50
48	52½												52

Weight is given to the nearest pound; height, to the nearest inch; age, to the nearest month. Up to and including 34 inches, weights are net; above this the following amounts have been added for clothing (shoes and sweaters not included): 35–39 inches, 1 pound; 40–44 inches, 1½ pounds; 45–49 inches, 1¾ pounds.

TABLE 16. Weight–Height–Age Table for Boys of School Age. From Baldwin and Wood (23)

Ht., in.	Av. wt. for ht., lbs.	Age, years															Ht., in.
		5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
38	34	34	34														38
39	35	35	35														39
40	36	36	36														40
41	38	38	38	38													41
42	39	39	39	39	39												42
43	41	41	41	41	41												43
44	44	44	44	44	44												44
45	46	46	46	46	46	46											45
46	48	47	48	48	48	48											46
47	50	49	50	50	50	50	50										47
48	53		52	53	53	53	53										48
49	55		55	55	55	55	55	55									49
50	58		57	58	58	58	58	58	58								50
51	61			61	61	61	61	61	61								51
52	64			63	64	64	64	64	64	64							52
53	68			66	67	67	67	67	68	68							53
54	71				70	70	70	70	71	71	72						54
55	74				72	72	73	73	74	74	74						55
56	78				75	76	77	77	77	78	78	80					56
57	82					79	80	81	81	82	83	83					57
58	85					83	84	84	85	85	86	87					58
59	89						87	88	89	89	90	90	90				59

Ht., in.	Av. wt. for ht., lbs.	Age, years															Ht., in.
		5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
60	94						91	92	92	93	94	95	96				60
61	99							95	96	97	99	100	103	106			61
62	104							100	101	102	103	104	107	111	116		62
63	111							105	106	107	108	110	113	118	123	127	63
64	117								109	111	113	115	117	121	126	130	64
65	123								114	117	118	120	122	127	131	134	65
66	129									119	122	125	128	132	136	139	66
67	133									124	128	130	134	136	139	142	67
68	139										134	134	137	141	143	147	68
69	144										137	139	143	146	149	152	69
70	147										143	144	145	148	151	155	70
71	152										148	150	151	152	154	159	71
72	157											153	155	156	158	163	72
73	163											157	160	162	164	167	73
74	169											160	164	168	170	171	74
Age, years			6	7	8	9	10	11	12	13	14	15	16	17	18	19	
Average height, inches																	
Short			43	45	47	49	51	53	54	56	58	60	62	64	65	65	
Medium			46	48	50	52	54	56	58	60	63	65	67	68	69	69	
Tall			49	51	53	55	57	59	61	64	67	70	72	72	73	73	
Average annual gain, lbs.																	
Short			3	4	5	5	5	4	8	9	11	14	13	7	3		
Medium			4	5	6	6	6	7	9	11	15	11	8	4	3		
Tall			5	7	7	7	7	8	12	16	11	9	7	3	4		

TABLE 17. Weight-Height-Age Table for Girls of School Age. From Baldwin and Wood (23)

Ht., in.	Av. wt. for ht., lbs.	Age, years														Ht., in.
		5	6	7	8	9	10	11	12	13	14	15	16	17	18	
38	33	33	33													38
39	34	34	34													39
40	36	36	36	36												40
41	37	37	37	37												41
42	39	39	39	39												42
43	41	41	41	41	41											43
44	42	42	42	42	42											44
45	45	45	45	45	45	45										45
46	47	47	47	47	48	48										46
47	50	49	50	50	50	50	50									47
48	52		52	52	52	52	53	53								48
49	55		54	54	55	55	56	56								49
50	58		56	56	57	58	59	61	62							50
51	61			59	60	61	61	63	65							51
52	64			63	64	64	64	65	67							52
53	68			66	67	67	68	68	69	71						53
54	71				69	70	70	71	71	73						54
55	75				72	74	74	74	75	77	78					55
56	79					76	78	78	79	81	83					56
57	84					80	82	82	82	84	88	92				57
58	89						84	86	86	88	93	96	101			58
59	95						87	90	90	92	96	100	103	104		59

Ht., in.	Av. wt. for ht., lbs.	Age, years														Ht., in.
		5	6	7	8	9	10	11	12	13	14	15	16	17	18	
60	101						91	95	95	97	101	105	108	109	111	60
61	108							99	100	101	105	108	112	113	116	61
62	114							104	105	106	109	113	115	117	118	62
63	118								110	110	112	116	117	119	120	63
64	121								114	115	117	119	120	122	123	64
65	125								118	120	121	122	123	125	126	65
66	129									124	124	125	128	129	130	66
67	133									128	130	131	133	133	135	67
68	138									131	133	135	136	138	138	68
69	142										135	137	138	140	142	69
70	144										136	138	140	142	144	70
71	145										138	140	142	144	145	71
Age, years			6	7	8	9	10	11	12	13	14	15	16	17	18	
Average height, inches																
Short			43	45	49	49	50	52	54	57	59	60	61	61	61	
Medium			45	47	52	52	54	56	58	60	62	63	64	64		
Tall			47	50	53	55	57	59	62	64	66	66	67	67		
Average annual gain, lbs.																
Short			4	4	4	5	6	6	10	13	10	7	2	1		
Medium			5	6	6	7	8	10	13	10	6	4	3	1		
Tall			6	8	8	9	11	13	9	8	4	4	1	1		

the measured heat production should first be referred to weight standards and then to height standards. If there is a substantial difference between them, the basal metabolic rate should be computed on the basis of standards referring to height; and since the height standards are based on a normal weight, they can be called standards for "expected weight."

TABLE 18A

Standard Total Calories for Height* (or Total Calories for Expected Weight)

Height, cm.	Total Calories per 24 hours		Height, cm.	Total Calories per 24 hours	
	Girls	Boys		Girls	Boys
48	134	—	92	681	725
50	159	—	94	695	740
51	—	160	96	709	755
52	186	175	98	722	765
54	214	200	100	735	785
56	246	225	105	770	805
58	278	260	110	807	830
60	309	300	115	846	875
62	341	315	120	894	935
64	373	360	125	942	990
66	404	390	130	987	1,045
68	433	420	135	1,057	1,105
70	462	450	140	1,130	1,165
72	489	480	145	1,208	1,220
74	515	510	150	1,294	1,290
76	539	535	155	1,386	1,380
78	560	565	160	1,477	1,480
80	581	590	165	1,544	1,570
82	601	612	170	1,584	1,655
84	619	635	175	1,596	1,720
86	636	660	180	1,600	1,800
88	652	685	190	—	1,900
90	666	705			

From Talbot (24).

* Since the height standard is based on a normal weight, this can also be called expected weight.

Two groups of standards referring to body size will be considered in the discussion that follows. The first is total Calories for height and weight (Tables 18A, 18B), as established by Talbot, which must be used with the height–weight tables of Woodbury (Tables 14, 15) and of Baldwin and Wood (Tables 16, 17). The second group is total Calories for height, weight, and surface area as established by Lewis, Duval, and Iliff (17) (Tables 19–21), and which are to be used with the height–weight tables established by them (Table 22).

TABLE 18B
Standard Total Calories for Weight

Weight, Kg.	Total Calories per 24 hours		Weight, Kg.	Total Calories per 24 hours	
	Girls	Boys		Girls	Boys
3	136	150	36	1,173	1,270
4	205	210	38	1,207	1,305
5	274	270	40	1,241	1,340
6	336	330	42	1,274	1,370
7	395	390	44	1,306	1,400
8	448	445	46	1,338	1,430
9	496	495	48	1,369	1,460
10	541	545	50	1,399	1,485
11	582	590	52	1,420	1,505
12	620	625	54	1,458	1,555
13	655	665	56	1,487	1,580
14	687	700	58	1,516	1,600
15	718	725	60	1,544	1,630
16	747	750	62	1,572	1,660
17	775	780	64	1,599	1,690
18	802	810	66	1,626	1,725
19	827	840	68	1,653	1,765
20	852	870	70	1,679	1,785
22	898	910	72	1,705	1,815
24	942	980	74	1,731	1,845
26	984	1,070	76	1,756	1,870
28	1,025	1,100	78	1,781	1,900
30	1,063	1,140	80	1,805	—
32	1,101	1,190	82	1,830	—
34	1,137	1,230	84	1,855	2,000

From Talbot (24).

B.M.R. Computed with Standards Referring to Urinary Creatinine. Talbot's "creatinine standards" (18) are based upon the premise that the amount of preformed creatinine excreted in the urine is a very exact index of the muscle mass; since this mass constitutes an almost constant proportion of the protoplasmic mass accounting for basal heat production, it follows that the excreted creatinine is also an index of the amount of active, heat-producing tissue (19). Total heat production can therefore be referred to urinary creatinine instead of to weight, height, or body surface.

Standards have been established by measuring creatinine excretion and basal caloric output in a large number of normal children between the ages of 5 and 14 years (Table 23). For this method of reference, the creatinine excretion must be measured (page 170) for 3 consecutive days before the metabolism test.

TABLE 19
Standard Values for Calories per Hour Referred to Weight

Weight, Kg.	Calories per hour		Weight, Kg.	Calories per hour	
	Boys	Girls		Boys	Girls
10	—	24.3	30	47.6	45.8
11	—	26.2	31	48.3	46.7
12	30.5	28.0	32	49.0	47 3
13	31.9	29.8	33	49.6	48.0
14	33.2	31.2	34	50.3	48.5
15	34.5	32.7	35	51.0	49.0
16	35.7	34.0	36	51.7	49.5
17	37.0	35.2	37	52.4	50.0
18	38.2	36.1	38	53.1	50.5
19	39.2	37.2	39	53.9	51.0
20	40.2	38.0	40	54.6	51.7
21	41.2	39.0	41	55.4	52 5
22	42.1	39.8	42	56.2	53.5
23	43.0	40.5	43	57.0	54.6
24	43.8	41.2	44	57.8	56.0
25	44.5	42.0	45	58.6	—
26	45.2	42.7	46	59.4	—
27	45.8	43.5	47	60.3	—
28	46.4	44.4	48	61.2	—
29	47.0	45.2	49	62.0	—
			50	62.8	—

From Lewis, Duval, and Iliff (17).

By using the creatinine standards, one avoids the much disputed choice of standards referred to physical development. Although creatinine output also is affected by variables other than mass of muscle tissue (20), such variations do not essentially change the constant relationship between basal metabolism and creatine excretion. Computation of the basal metabolic rate by means of creatinine standards is simple and reliable; eventually it may prove to be a better method than any other for computing the basal metabolic rate in children.

PROCEDURE

Up to 14 hours before the test the child is allowed his usual diet; thereafter, neither food nor drink until after the test. If the test is to be performed in the morning, no food or drink should be given after the previous evening meal.

TABLE 20

Standard Values for Calories per Hour Referred to Height

Height, cm.	Calories per hour		Height, cm.	Calories per hour	
	Boys	Girls		Boys	Girls
84	30.7	26.8	130	45.3	43.0
86	—	27.5	132	46.0	43.8
88	30.7	28.2	134	46.6	44.6
90	31.4	28.9	136	47.2	45.4
92	32.1	29.6	138	47.9	46.2
94	32.8	30.3	140	48.6	47.0
96	33.5	31.0	142	49.3	47.8
98	34.2	31.7	144	50.0	48.7
100	34.9	32.4	146	50.7	49.6
102	35.6	33.1	148	51.6	50.5
104	36.3	33.8	150	52.5	51.4
106	37.0	34.5	152	53.5	52.3
108	37.7	35.2	154	54.9	53.2
110	38.4	35.9	156	56.3	54.1
112	39.1	36.6	158	57.7	55.0
114	39.8	37.3	160	59.1	55.9
116	40.5	38.0	162	60.5	56.8
118	41.2	38.7	164	61.9	57.7
120	41.8	39.4	166	63.2	58.6
122	42.5	40.1	168	64.3	—
124	43.2	40.8	170	65.3	—
126	43.9	41.5	172	66.0	—
128	44.6	42.2	174	66.6	—

From Lewis, Duval, and Iliff (17).

The age, weight, and height of the child are noted.

The child must lie down for at least 30 minutes before the test is started, and the most complete relaxation must be striven for. There should be absolute quiet in adjacent rooms. Whether the mother or nurse should remain in the room with the child must be decided as the situation demands.

Pulse rate and temperature are taken at the end of the rest period. If the pulse rate is more than 15 beats per minute above

TABLE 21

Standard Values for Calories per Hour Referred to Surface Area

Surface area, sq. m.	Calories per hour		Surface area, sq. m.	Calories per hour	
	Boys	Girls		Boys	Girls
0.500	—	26.2	1.060	47.1	45.2
.520	—	27.0	.080	47.6	45.8
.540	30.8	28.1	.100	48.1	46.4
.560	31.6	29.5	.120	48.5	47.0
.580	32.4	30.4	.140	49.0	47.6
600	33.1	31.3	.160	49.5	48.2
.620	33.8	32.2	.180	50.0	48.8
.640	34.5	33.0	.200	50.4	49.4
.660	35.2	33.8	.220	51.0	49.9
.680	35.9	34.5	.240	51.7	50.5
.700	36.6	35.2	.260	52.5	51.1
.720	37.3	35.8	.280	53.3	51.6
.740	38.0	36.4	.300	54.1	52.2
.760	38.7	37.0	.320	54.8	52.7
.780	39.4	37.6	.340	55.5	53.3
.800	40.1	38.2	.360	56.2	53.9
.820	40.7	38.8	.380	56.9	54.5
.840	41.3	39.4	.400	57.6	55.1
.860	42.0	39.9	.420	58.3	55.6
.880	42.6	40.4	.440	59.0	56.2
.900	43.2	40.9	.460	59.7	56.8
.920	43.8	41.4	.480	60.4	47.5
.940	44.4	41.9	.500	61.1	57.9
.960	45.0	42.4	.520	61.8	58.5
.980	45.5	42.9	.540	62.5	59.1
1.000	46.0	43.4	.560	—	59 7
1.020	46.3	44.0	.580	—	60.3
1.040	46.7	44.6	—	—	—

From Lewis, Duval, and Iliff (17).

normal (page 93), or the temperature is over 99.5 F. (37.5 C.), the test must be postponed. This does not apply to children suffering from cardiovascular ailments.

The nose clip is then adjusted, and the child is allowed to adapt himself to the breathing procedure. There should be no escape of air through the nostrils when the mouthpiece is introduced. The test is then performed by the routine method for the apparatus used, and the oxygen consumption determined during a unit of time. Most of the portable apparatus record the respiration graphically; if this is irregular in depth, the test must be rejected. Occasionally,

TABLE 22

Mean, Maximum, and Minimum Values for Weight, Height, and Surface Area Computed with Dubois and Dubois Height–Weight Formula with Constant 71.84

Age, years	Weight, Kg.			Height, cm.			Surface area, sq. m.		
	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.
				Boys					
2–3	13.7	16.4	12.0	93.0	98.5	86.9	0.584	0.645	0.525
3–4	15.7	19.4	12.9	100.1	107.3	93.7	0.653	0.750	0.590
4–5	17.7	22.2	14.5	107.7	119.4	101.0	0.724	0.860	0.640
5–6	19.8	24.7	15.9	114.5	128.3	105.7	0.794	0.945	0.690
6–7	22.6	29.6	16.7	121.6	134.2	113.0	0.875	1.040	0.730
7–8	25.4	34.8	18.3	127.6	136.8	116.2	0.950	1.150	0.800
8–9	28.0	40.8	20.5	132.8	143.8	119.4	1.019	1.275	0.855
9–10	30.1	45.2	22.3	137.1	148.5	125.3	1.077	1.360	0.900
10–11	32.5	45.7	23.9	141.6	152.4	128.5	1.140	1.390	0.950
11–12	35.6	54.4	25.9	146.8	159.0	133.3	1.216	1.550	1.010
12–13	39.0	60.2	28.3	151.4	170.1	137.3	1.293	1.695	1.080
13–14	44.0	64.3	31.3	156.6	176.2	142.5	1.392	1.790	1.155
14–15	48.5	67.2	37.4	163.9	178.2	146.9	1.503	1.830	1.270
15–16	53.0	65.9	45.0	170.3	178.0	154.7	1.607	1.800	1.395
				Girls					
2–3	13.0	15.5	10.1	92.0	100.9	81.0	0.566	0.640	0.465
3–4	15.2	17.8	12.0	100.5	109.8	93.4	0.647	0.735	0.545
4–5	17.3	20.8	12.6	107.4	118.7	98.8	0.715	0.830	0.595
5–6	19.6	24.7	14.2	114.4	125.3	104.1	0.788	0.915	0.645
6–7	21.9	29.8	15.5	120.7	131.5	110.8	0.860	1.030	0.715
7–8	24.9	34.1	16.9	127.6	139.0	116.4	0.943	1.150	0.750
8–9	28.5	42.1	17.5	133.0	145.4	119.3	1.029	1.280	0.805
9–10	32.1	44.4	19.5	139.0	153.7	126.1	1.119	1.350	0.845
10–11	34.7	43.3	21.2	144.5	155.7	130.1	1.190	1.380	0.895
11–12	40.2	50.7	27.2	151.7	164.2	136.2	1.310	1.515	1.025
12–13	43.4	54.7	30.4	155.4	168.5	141.3	1.376	1.555	1.110
13–14	49.5	59.6	35.5	161.5	170.7	148.3	1.499	1.650	1.230
14–15	54.8	64.1	39.7	164.2	171.9	155.3	1.583	1.685	1.325
15–16	55.4	65.3	43.9	167.8	173.5	160.5	1.620	1.750	1.420

From Lewis, Duval, and Iliff (17).

more than 2 test periods are necessary to obtain 2 readings which do not differ by more than 5 per cent.

Calculation. The mean average volume of oxygen consumed during the test period is expressed in liters per hour and liters per 24 hours. These figures are corrected to standard temperature (0 C.) and standard pressure (760 mm. Hg) by means of tables. The newer portable apparatus register the corrected figure, so that no further correction is necessary. The value of oxygen consumed per hour thus obtained is converted into an equivalent caloric value by multiplying by 4.8, under the assumption that the respiratory quotient is 0.83. This caloric value (total Calories) represents the child's basal heat production; any report on basal metabolism should give this information on total heat production per hour.

The figure representing total Calories is now compared with the corresponding figure for the normal child as found in standards tables, referred (*1*) to weight and height, or (*2*) body surface area, or (*3*) creatinine output.

With Standards Referred to Weight and Height. The most commonly used standards are those of F. B. Talbot, which cover the entire childhood period. In connection with these, the weight–height tables of Baldwin and Wood, and of Woodbury, must be used to assess body size.

Compare the patient's weight with the normal weight for height (Tables 14–17), and decide whether the child is of normal or abnormal body size (page 67). If the child's weight is normal, use Table 18B. The figure for total Calories per 24 hours is compared with the standard for the child's actual weight, and the deviation from the standard is expressed as plus or minus per cent. This figure is the basal metabolic rate.

If the child is of abnormal body size, use Table 18A, which refers total Calories to height instead of to weight, and calculate as above.

Other standards referred to weight and height, for example, those of Lewis *et al.* (Tables 19–22), may be employed, provided they are used with the height-weight tables which belong to them. If the Lewis standards are used, the weight of the child is compared to the normal weight in Table 22. If the child is of normal body size, use Table 19 to compute basal metabolic rate; if body size is abnormal, use Table 20.

With Standards Referred to Body Surface Area. The Lewis tables give both the caloric standards and the normal values of body measurements. The patient's age, weight, height, and surface area must be known. Compute the surface area from Figure 7, and check the child's body measurements against the normal values, as given in Table 22. Decide whether the child is of normal or abnormal body size (page 67).

If the child is of normal body size, compare the total Calories per hour with the standard Calories in Table 21. The deviation from the standard is expressed as plus or minus per cent, and is the basal metabolic rate.

If body size is abnormal, use Table 20, which gives caloric standards referred to height, and proceed as above.

Any other table of caloric standards referred to surface area may be used in similar fashion, provided the standards are accompanied by their respective tables for normal height and weight.

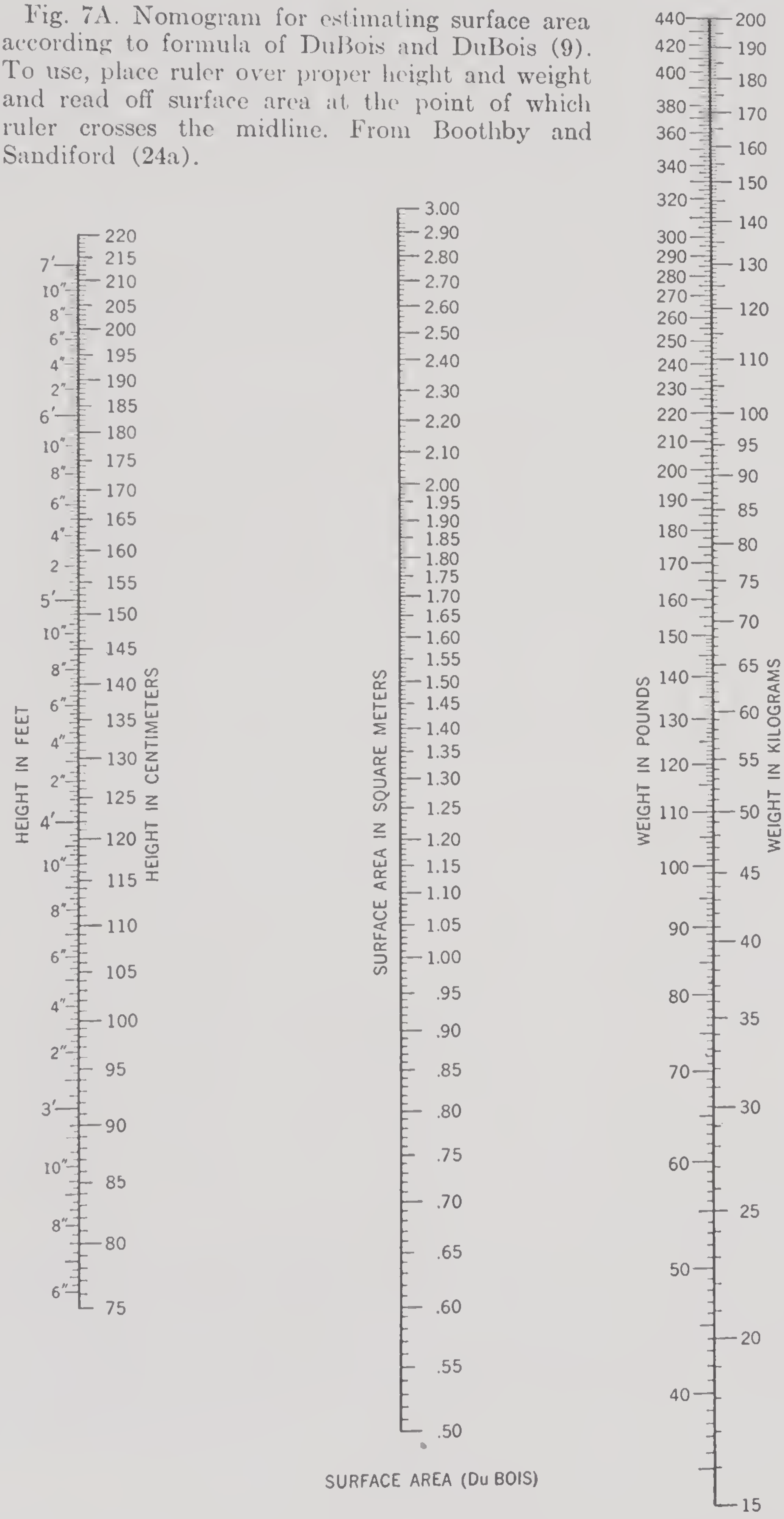
With Standards Referred to Creatinine Output. The only standards available are those of N. B. Talbot. The creatinine excretion (page 170) for three consecutive 24 hour periods is averaged, and expressed in milligrams. This represents the total creatinine output. Whether the creatinine output is relatively high or low is unimportant. Compare the child's total Calories per 24 hours with standards given in Table 23, which lists the total calories normally

TABLE 23
Standard Total Calories Referred to Creatinine Output

Creatinine output, mg. per 24 hrs.	Total Calories per 24 hrs.		Creatinine output, mg. per 24 hrs.	Total Calories per 24 hrs.	
	Boys	Girls		Boys	Girls
350	1,052	969	850	1,381	1,298
400	1,085	1,002	900	1,414	1,331
450	1,118	1,035	950	1,446	1,363
500	1,151	1,068	1,000	1,479	1,396
550	1,184	1,101	1,050	1,512	1,429
600	1,216	1,133	1,100	1,545	1,462
650	1,249	1,116	1,150	1,578	1,495
700	1.282	1,199	1,200	1,611	1,528
750	1,315	1,232			
800	1,348	1,265	1,250	1,644	1,561

From Talbot, Worcester, and Stewart (21).

Fig. 7A. Nomogram for estimating surface area according to formula of DuBois and DuBois (9). To use, place ruler over proper height and weight and read off surface area at the point of which ruler crosses the midline. From Boothby and Sandiford (24a).



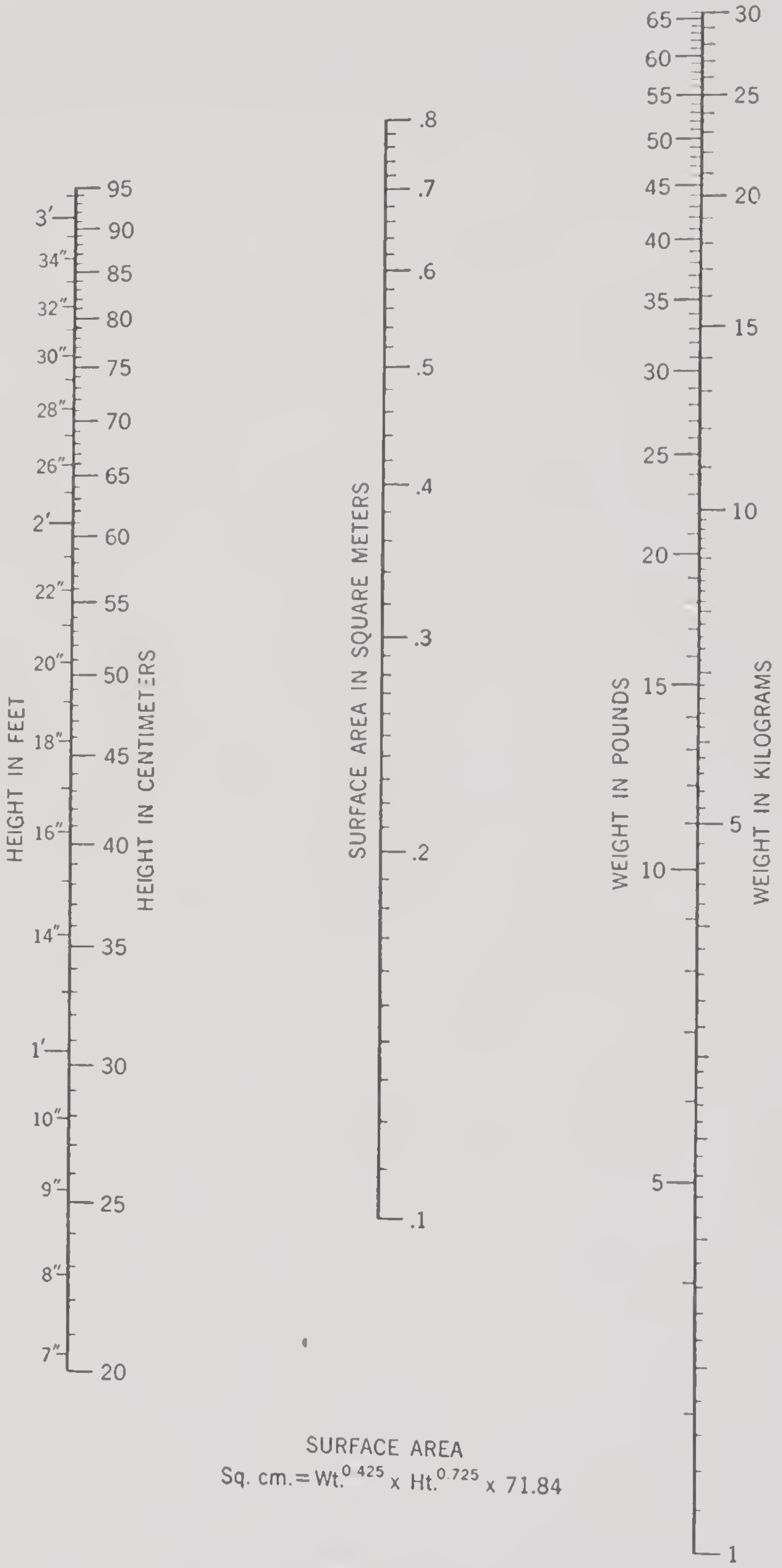


Fig. 7B. Nomogram for estimating surface area of persons weighing less than 20 kilograms according to formula of DuBois and DuBois (9). To use, place ruler over proper height and weight and read off surface area at the point at which ruler crosses the midline. From DuBois (6).

to be expected in a child excreting given amounts of creatinine. The deviation from the standard is expressed as plus or minus per cent, and is the basal metabolic rate.

INTERPRETATION

The normal range of the basal metabolic rate in children is from plus 15 per cent to minus 15 per cent. All values above or below this range are considered abnormal. Clinically, persistently high values (above +20 per cent) are a sign of an overactive thyroid gland. Generally, the hyperthyroidism is caused by disease of the thyroid gland, such as exophthalmic goiter, Graves' disease, or prepubertal hyperthyroidism. Occasionally, however, it may be the result of hyperfunction of the pituitary or adrenal glands.

A persistent basal metabolic rate of minus 20 per cent or lower suggests hypofunction of the thyroid, such as is found in cretinism or myxedema. Pluriglandular disorders involving the pituitary and adrenal glands may also cause hypothyroidism and a low metabolic rate. This is the case in pituitary dwarfism, Froehlich's syndrome, pluriglandular endocrine disturbances, Addison's disease, pituitary obesity, and amaurotic familial idiocy.

The clinical interpretation of metabolic rates which fall between plus or minus 15 and 20 is more difficult, and requires a prudent and critical approach. One should keep in mind that the basal metabolic rate and the degree of thyroid activity do not always parallel each other. Some patients, when judged by their metabolic rate, might be considered borderline cases; clinically, however, they present unmistakable, classic signs of endocrine or thyroid disorders. On the other hand, there are cases where even small, but consistent deviations from normal aid considerably in confirming the clinical impression of lowered thyroid activity. It is therefore wise never to base a diagnosis of borderline hypothyroid conditions upon a single functional test, not even the basal metabolism test.

The case records below illustrate the various methods of computing the basal metabolic rate. Reports on basal metabolism of children should include (*1*) the child's body measurements, (*2*) mention of the tables used for comparison of body measurements, (*3*) total calories produced per hour and per 24 hours, and (*4*) caloric standards used for computing the basal metabolic rate.

Case 1. New York Post-Graduate Hospital, No. J 80130, 1943, A.M., female. Clinical diagnosis: pituitary dwarfism.

Age: 14 years, 6 months
Weight: 57.5 pounds (26.1 Kg.)
Height: 40.3 inches (102.5 cm.)
Surface area: 0.85 square meter (computed from Fig. 7)
Oxygen adsorbed: 116.0 cc. per minute
Total Calories per hour: 33.4
Total Calories per 24 hours: 801.8
Total Calories per hour per square meter: 40.26

B.M.R. Computed with F. B. Talbot's Standards. Table 17 gives the range in height of a normal 14 year old girl as 59 to 66 inches, with an average of 59 inches for the short types. The patient's height is 40.3 inches, or 31.7 per cent below the average. Her weight is far below the normal range for her age, but referred to her actual height, it is 59.7 per cent above the "expected weight." The child is therefore of abnormal body size, and the standards for total Calories for "expected weight," i.e., standards referred to height, must be used. Table 18A gives 752.5 Calories as the standard for a girl 102.5 cm. tall; the total Calories determined in the girl were 801.8, i.e., 49.3 Calories, or 6.6 per cent above the standard. B.M.R. is therefore plus 6.6 per cent.

B.M.R. Computed with the Standards of Lewis et al. The patient's weight, height, and surface area are compared with the standards in Table 22, which gives for a 14½ year old girl a normal average weight of 54.8 kilograms, a height of 164.2 centimeters, and a surface area of 0.583 square meter. The girl's actual measurements, when compared with these standards, are minus 52.4 per cent in weight, minus 37.6 per cent in height, and minus 49.4 per cent in surface area. The child is therefore of abnormal body size, and caloric standards referred to height must be used. Table 20 gives 33.1 Calories per hour as the standard for a girl 102.5 centimeters tall. Total Calories per hour, as determined in the patient, are 33.4, i.e., 0.3 Calorie or 1 per cent above the standard. B.M.R. is therefore plus 1 per cent.

The child's basal metabolism, computed by two different standards, is normal.

Case 2. New York Post-Graduate Hospital, No. C 100299, 1943, L. T., male. Clinical diagnosis: constitutional obesity.

Age: 5 years, 3 months
Weight: 61.5 pounds (28.1 Kg.)

Height: 45 inches (114 cm.)
Surface area: 0.92 square meter (computed from Fig. 7)
Oxygen adsorbed: 154.2 cc. per minute
Total Calories per hour: 44.4
Total Calories per 24 hours: 1,067

B.M.R. Computed with F. B. Talbot's Standards. When compared with the figures in Table 16, it is found that the patient's height is high normal, but his weight is 35.4 per cent above the standard weight for his height ("expected weight," 45.5 lbs.; actual weight, 61.5 lbs.). The child is therefore of abnormal body size, and Table 18A must be used. For a boy 114 centimeters tall, the table gives a standard of 875 total Calories; the patient's total Calories are actually 1,067, i.e., 192 Calories or 21.9 per cent above standard. B.M.R. is plus 21.9 per cent.

B.M.R. Computed with Standards of Lewis et al. Table 22 gives a weight of 19.8 kilograms, a height of 114.5 centimeters, and a surface area of 0.794 square meter as the normal average measurements for a 5 year old boy. The patient's actual measurements, when compared with the standards, are plus 42 per cent in weight, normal in height, and plus 16 per cent in surface area. The child is therefore of abnormal body size, and Table 20 must be used. The standard value for a boy of 114 centimeter height is 39.8 Calories per hour. The patient's heat production is 44.4 Calories per hour, or 11.6 per cent above the normal value. B.M.R. is plus 11.6 per cent.

The results obtained with two different caloric standards show that the patient's basal metabolism is rather high, but not definitely abnormal. The patient may be classed as a borderline case.

Case 3. From Talbot *et al.* (21). Female child. Clinical diagnosis: hypothyroidism.

Age: 12 years, 6 months
Weight: 72.6 pounds (33 Kg.)
Height: 46.8 inches (119 cm.)
Surface area: 1.07 square meters
Total Calories per hour: 33.3
Total Calories per 24 hours: 800
Total Creatinine output: 410 milligrams (average of 3 periods of 24 hours)

B.M.R. Computed with Creatinine Standards. Table 23 gives a caloric value of 1,002 as normal with a creatinine excretion of

400 milligrams. The heat production established in the patient is 800 Calories, or 20.1 per cent less than the standard. B.M.R. is minus 20.1 per cent.

B.M.R. Computed with F. B. Talbot's Standards. Comparison of the patient's measurements with the standards given in Table 17 shows that the child is of abnormal body size. Table 18A must therefore be used. B.M.R. is minus 10.5 per cent.

B.M.R. Computed with Standards of Lewis et al. Comparison with the standards table (Table 22) shows that the child is of abnormal body size, and Table 20 must be used. B.M.R. is minus 13.9 per cent.

The patient's basal metabolism, as computed from the creatinine standard, is abnormally low. While it is also low, as computed from the other two standards, it is nevertheless within normal range. The patient must therefore be considered a borderline case, with a definite tendency to low basal metabolism.

DETERMINATION OF RESPIRATORY QUOTIENT

The term "respiratory quotient" (R.Q.) is applied to the ratio:

$$\frac{\text{Volume of } CO_2 \text{ output}}{\text{Volume of } O_2 \text{ output}}$$

in a given time. The ratio is not constant, changing with the character of the material metabolized. According to Richardson (25), "the respiratory quotient is the most direct measure, so far devised, of the relative quantity of foodstuffs entering into the metabolism of the human or animal organism."

When carbohydrates only are metabolized, the respiratory quotient is 1, since equal amounts of carbon dioxide and oxygen, respectively, are evolved and required. For example, the equation for the metabolism of glucose is:

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O$$
$$\text{R.Q.} = 6\,CO_2/6\,O_2 = 1$$

When fat is the only material metabolized, more oxygen is needed, and the respiratory quotient is reduced to about 0.7. The equation for tristearin, for instance, is:

$$C_{56}H_{110}O_6 + 81.5\,O_2 \rightarrow 57\,CO_2 + 55\,H_2O$$
$$\text{R.Q.} = 57/81.5 = 0.699$$

Oxidation of protein alone yields a respiratory quotient of about 0.8. Since no empiric formula for a protein is available the ratio has been calculated on the basis of urinary nitrogen elimination (26).

Healthy individuals, on a normally mixed diet, utilize a metabolic mixture composed of all three foodstuffs. The resulting respiratory exchange is characterized by a respiratory quotient of 0.82 to 0.85. A rise above this level toward unity is a sign that carbohydrate, rather than fat or protein, is being metabolized. Conversely, when the respiratory quotient falls below 0.82 and approaches 0.7, the predominance of fat combustion is revealed.

Respiratory quotients calculated from the total oxygen consumption and the total carbon dioxide production are termed "total R.Q." in distinction to the "nonprotein R.Q.," a designation applied to the ratio:

$$\frac{\text{Nonprotein } CO_2 \text{ production}}{\text{Nonprotein } O_2 \text{ consumption}}$$

Some authors believe that determination of nonprotein respiratory quotient is not essential for clinical purposes, since the total quotient is only slightly altered by making allowance for the metabolism of protein (27). However, the nonprotein respiratory quotient is indispensable for quantitative answers as to heat production from carbohydrate and fat. Only with these figures in hand can one determine from Zuntz's table (Table 24): (*1*) the caloric equivalent of a liter of oxygen, and (*2*) the exact percentages of carbohydrate and fat that are undergoing combustion.

PEDIATRIC CONSIDERATIONS

The technical problems involved in determining the respiratory quotient in children are of the same kind as in the basal metabolism test. Since apparatus are needed which allow measurement of both oxygen intake and carbon dioxide output, the simpler type of machines, such as the Benedict-Roth respirator, cannot be used; they measure oxygen consumption only. The apparatus needed are: (*1*) respiration chambers, as part of a closed-circuit type system, for testing infants and smaller children; (*2*) for children 6 years of age and older, one of the closed or open-circuit type respiration apparatus, the patient being connected with the system by tubing and a mouthpiece; *(3)* gas analysis equipment.

In practice, these technical requisites, as well as the necessary trained personnel, inevitably restrict the use of this method. The respiratory quotient of infants and young children can be investigated in only a small number of institutions.

The respiratory exchange may be determined during various metabolic phases. In the following discussion, the determination and significance of the respiratory exchange will be considered when obtained *(1)* under basal conditions, i.e., resting or basal respiratory quotient; *(2)* prior to and after varying periods of fasting; and *(3)* prior to and after ingestion of carbohydrates. The last two determinations are of particular value in appraising the severity of the metabolic imbalance in diabetes (28), in detecting a disposition to ketosis (29), and in evaluating the various factors to which changes in the respiratory quotient may be ascribed (30).

PROCEDURE

One of the open-circuit type methods (page 65) is used for children 6 years of age or older. The subject breathes atmospheric air through valves which separate the inspired from the expired air, and the expired air is collected into a Tissot spirometer or Douglas bag. At the end of a minimum 5 minute breathing period, the total volume of expired air is measured and samples are analyzed for oxygen and carbon dioxide content by means of the Haldane-Henderson or Van Slyke apparatus. Details of the procedure may be found in any one of the comprehensive studies on respiratory exchange (31–33).

A 4 hour urine sample should be collected from the subject prior to the test, and its nitrogen content ascertained, for instance, by the method outlined on page 488.

Calculation. Best and Taylor (33) give as an example a total oxygen consumption of 16 liters per hour, a total carbon dioxide production of 13.5 liters per hour, and a urinary nitrogen elimination of 0.5 Gm. per hour; the total respiratory quotient is: 13.5/16 = 0.844.

According to Lusk, 1 Gm. of urinary nitrogen is equivalent to 6.25 Gm. of protein, and represents the absorption of 5.94 liters of

TABLE 24

Analysis of Oxidation of Mixtures of Carbohydrate and Fat. From Lusk (34)

R.Q.	Percentage total oxygen consumed by		Percentage total heat produced by		Calories per liter O_2	
	Carbohydrate (1)	Fat (2)	Carbohydrate (3)	Fat (4)	Number (5)	Logarithm (6)
0.707	0	100.0	0	100.0	4.686	0.67080
0.71	1.02	99.0	1.10	98.9	4.690	0.67114
0.72	4.44	95.6	4.76	95.2	4.702	0.67228
0.73	7.85	92.2	8.40	91.6	4.714	0.67342
0.74	11.3	88.7	12.0	88.0	4.727	0.67456
0.75	14.7	85.3	15.6	84.4	4.739	0.67569
0.76	18.1	81.9	19.2	80.8	4.751	0.67682
0.77	21.5	78.5	22.8	77.2	4.764	0.67794
0.78	24.9	75.1	26.3	73.7	4.776	0.67906
0.79	28.3	71.7	29.9	70.1	4.788	0.68018
0.80	31.7	68.3	33.4	66.6	4.801	0.68129
0.81	35.2	64.8	36.9	63.1	4.813	0.68241
0.82	38.6	61.4	40.3	59.7	4.825	0.68352
0.83	42.0	58.0	43.8	56.2	4.838	0.68463
0.84	45.4	54.6	47.2	52.8	4.850	0.68573
0.85	48.8	51.2	50.7	49.3	4.862	0.68683
0.86	52.2	47.8	54.1	45.9	4.875	0.68793
0.87	55.6	44.4	57.5	42.5	4.887	0.68903
0.88	59.0	41.0	60.8	39.2	4.899	0.69012
0.89	62.5	37.5	64.2	35.8	4.911	0.69121
0.90	65.9	34.1	67.5	32.5	4.924	0.69230
0.91	69.3	30.7	70.8	29.2	4.936	0.69339
0.92	72.7	27.3	74.1	25.9	4.948	0.69447
0.93	76.1	23.9	77.4	22.6	4.961	0.69555
0.94	79.5	20.5	80.7	19.3	4.973	0.69663
0.95	82.9	17.1	84.0	16.0	4.985	0.69770
0.96	86.3	13.7	87.2	12.8	4.998	0.69877
0.97	89.8	10.2	90.4	9.58	5.010	0.69984
0.98	93.2	6.83	93.6	6.37	5.022	0.70091
0.99	96.6	3.41	96.8	3.18	5.035	0.70197
1.00	100.0	0	100.0	0	5.047	0.70303

Formula for column:*

(1) $\% = 100\,\dfrac{R - 0.707}{0.293}$ (2) $\% = 100\,\dfrac{1.00 - R}{0.293}$

(3) $\% = \dfrac{504.7\,(R - 0.707)}{5.047\,(R - 0.707) + 4.686\,(1.00 - R)}$

(4) $\% = \dfrac{468.6\,(1.00 - R)}{5.047\,(R - 0.707) + 4.686\,(1.00 - R)}$

(5) $\text{Calories} = 4.686 + \dfrac{(R - 0.707)\,0.361}{0.293}$ (6) Logarithm = log column 5

* R.2. = R in formulas above.

oxygen and the elimination of 4.76 liters of carbon dioxide. The catabolism of protein in the above example, therefore, accounts for 0.5 × 4.76, or 2.38 liters of carbon dioxide produced, and 0.5 × 5.94, or 2.97 liters of oxygen consumed. By substracting these figures from the total volumes, one obtains the nonprotein respiratory quotient by the formula:

$$\frac{13.5 - 2.38 \text{ L. } CO_2}{16.0 - 2.97 \text{ L. } O_2} = \frac{11.2}{13.03} = 0.853$$

For nonprotein respiratory quotients ranging from 0.707 to 1.0, the percentage of fat and carbohydrate accounting for the nonprotein combustion can be read from Table 24.

Although allowance for protein catabolism does not alter the total respiratory quotient significantly, most clinical studies adhere to the calculation of the nonprotein respiratory quotient.

INTERPRETATION

The following tabulation from Cantarow and Trumper (35) may serve as a guide in analyzing the respiratory quotient resulting from the combustion of different substances.

Substance metabolized	Respiratory quotient
Carbohydrates chiefly	1
Fat chiefly	0.7
Protein chiefly	0.8
Fat converted to carbohydrate	0.7–0.6
Carbohydrate converted to fat	1.0–1.3

The respiratory quotient of healthy individuals on a normal diet for their age is around 0.82 in the postabsorptive state (basal R.Q.), and around 0.85 in the absorptive state. Except in the newborn, these figures are also considered normal in infants and children. The respiratory quotient approaches unity during the first hours of life, drops to 0.7 to 0.72 at the end of the first day, and then gradually rises until the fifth or sixth day of life it has reached the average normal of 0.82 (37).

High respiratory quotients, when they are the result of true metabolic disturbances, indicate that carbohydrates chiefly are being metabolized. Such clinical conditions do not occur spontaneously, but may be provoked by a carbohydrate diet, by the first phase of violent exercise, or by epinephrine administration. The increase in the respiratory quotient observed in any form of rapidly developing acidosis (except in exercise) is nonmetabolic in nature, for it is not associated with a corresponding rise in oxygen consumption. Elevated respiratory quotients are obtained during convalescence (38).

Low respiratory quotients indicate that fat mainly is being metabolized. Such quotients are obtained in diabetes and in hyperthyroidism. In diabetes, the basal respiratory quotient is usually between 0.7 and 0.72; the lower the quotient, the more severe is the impairment of carbohydrate utilization (39). In hyperthyroidism, the low quotient is due to depletion of glycogen reserves. The decrease in the respiratory quotient in clinical conditions associated with severe alkalosis is nonmetabolic in nature. During hyperventilation or recovery from severe acidosis, for example, the fall results from carbon dioxide retention to form bicarbonate (40).

Response of Respiratory Quotient to Dietary Provocation. The respiratory quotient may be raised by the administration of various carbohydrates. The oral ingestion of one of the monosaccharides or disaccharides (1.75 Gm./Kg. body weight) leads to an increased respiratory quotient varying in degree with the sugar ingested. In adults, according to Edwards *et al.* (30), "the greatest increases in the R.Q. are after fructose ingestion. This implies that there is a more rapid combustion of carbohydrate after fructose than after glucose or galactose." The results in older children are, as a rule, comparable to those obtained in adults. Law and Gay (41) measured the respiratory quotient in an 11 year old girl at intervals of ½, 1, 2, 3, and 4 hours after ingestion of various sugars in a dosage of 1.75 Gm. per kilogram of body weight. They found that the respiratory quotient increased most rapidly after sucrose ingestion; for the other sugars, the increase was, in descending order: galactose, fructose, dextrimaltose, lactose, and dextrose. With the last mentioned, the respiratory quotient rose to 0.92, as compared with an average normal rise to 0.96 in adults.

In diabetes the increase in the respiratory quotient following glucose ingestion is abnormally low or absent, whereas in hyperthyroidism the rise is unusually high and rapid.

Ingestion of dextrose will fail to raise the low respiratory quotient of the fasting state, apparently because the sugar is being stored as glycogen (42).

The respiratory quotient may be lowered by a fat (ketogenic) diet, or by prolonged fasting (starvation). Only a slight decline is observed in the majority of normal children as a reaction to fasting (page 153). An abrupt fall to 0.7 or less suggests a lack of serviceable carbohydrate and/or an abnormally high tendency to metabolize fat and protein. Such abnormal reactions are usually combined with an increased tendency to develop ketosis, and are encountered in children with diabetes, cyclic vomiting, glycogen disease (Fig. 25, page 153), and hypofunction of the adrenal cortex (page 154).

TABLE 24A
Average Resting Pulse Rate in Healthy Children

Age	Rate per minute
Birth – 3 months	140–130
4–12 months	130–120
1–2 years	120–115
2–8 years	100–90
8–12 years	90–80
12–16 years	80–76

After P. Nobécourt and L. Babonneix (43).

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CHAPTER IV

Carbohydrate Metabolism Tests

Carbohydrates are the most important of the food constituents serving as sources of energy. Useful heat and energy are liberated by the breakdown of sugar molecules and the bonds of their compounds. Carbohydrates not immediately utilized are stored in the liver, and to a small extent in the muscles, in the form of glycogen, and it is upon these depots that the organism draws continuously for the sugar it needs. This sugar is released into the blood stream for transportation to the tissues only in the form of glucose.

These few facts, so briefly summarized, form the basis for the most important phases of carbohydrate metabolism, which are: (*1*) intestinal digestion of polysaccharides; (*2*) intestinal absorption of monosaccharides and their transportation to the liver; (*3*) anabolism and storage of glycogen in the liver; (*4*) reconversion (mobilization) of glycogen into glucose and its transport to the extrahepatic tissues; and (*5*) utilization of glucose by tissue cells.

The rate at which the liver releases sugar, and its utilization by the tissues, are in remarkable equilibrium under normal conditions. The miraculous constancy of the blood sugar level under varied conditions of activity and nutrition is now well established, and it is generally accepted that the regulation of this dynamic balance, which according to Soskin (1) "is governed by opposing forces of the pancrease on the one hand, and of the anterior pituitary, thyroid, adrenal cortex on the other," lies in the vegetative centers of the central nervous system.

Attempts to assess the functional efficiency of carbohydrate metabolism have of necessity centered upon the determination of the blood sugar level. Hyperglycemia and hypoglycemia in a fasting state, when observed repeatedly, are important signs of metabolic disease, and they frequently furnish the clue to the diagnosis. A change in blood sugar level, however, reveals nothing as to the particular phase—absorptive, assimilative or storing, mobilizing or utilizing—which is impaired.

The group of tolerance tests designed for a more functional examination of carbohydrate metabolism further aids diagnosis. Thus, the rise in the blood level of the particular sugar and the rate of its removal from the blood after oral or intravenous administration of a standard dose of glucose, levulose, or galactose is determined. An abnormally high and prolonged rise of the tolerance curve and the appearance of melituria indicate impaired capacity to dispose of the test dose of carbohydrate. The mechanism of the tolerance curve is a complex one; each of the metabolic phases mentioned above affects the curve's shape, and even when the absorptive phase and the renal sugar threshold can be ruled out as the factors responsible, the cause of an apparent intermediary imbalance is still not revealed. A pathologic response may be due to impaired liver or pancreatic function; it may indicate an abnormal rate of supply from the liver or of utilization by the tissues.

There is a third group of tests for investigating the mechanism of such abnormalities. This group measures the response of the blood sugar level to administration of endocrine preparations, such as epinephrine, insulin, or pituitrin. An unusually low rise in the blood sugar following epinephrine administration, for example, is definite indication that sugar formation from liver glycogen is impaired. As a supplement to these functional tests, the determination of the glycogen level in the blood is a valuable help in the differential diagnosis of diseases of sugar metabolism.

A fourth approach to the analysis of disorders of carbohydrate metabolism is to test the individual's tendency to develop ketosis. For a description of this method, see page 148.

Finally, some information can be gained by determining the respiratory quotient (page 87).

Examination of urine for sugar and ketone bodies is an essential part of any test. True glycosuria occurs when the blood level of glucose exceeds the renal glucose threshold. Consideration of other possible causes of melituria should never be omitted; these may be rest-reduction, pentosuria, galactosuria, levulosuria, renal diabetes, or drug administration.

The use of blood sugar tolerance tests in children has markedly increased since micromethods for blood glucose, levulose, and galactose determination have been devised which require only 0.1 to 0.2 cc. of blood for each determination. There are no technical difficulties any longer in securing the necessary series of blood samples from infants or small children in the course of a few hours.

BLOOD SUGAR ASSAYS

A number of micromethods have been recommended for use in children, among them the two described below—Folin's micromethod, and Reiner's modification of the Folin-Wu method—and the procedures of Hagedorn and Jensen (2) and of Boyd (3).

It should be noted that the "apparent" blood sugar, as determined by any of these methods, consists of glucose and of smaller or greater quantities of the nonfermentable reducing substances in the blood, such as creatinine, uric acid, glutathione, and ergothioneine. The amount of reduction not due to glucose depends on the precipitant chosen for removing the proteins, and it follows that the normal range of apparent sugar varies with the method employed (Table 25). Folin's micromethod, for example, gives values about 10 to 15 milligrams per hundred cubic centimeters lower than those obtained by the Folin-Wu macromethod or by Reiner's micromodification of it. The nearest estimate of "true" blood sugar is attained by removing the proteins by Somogyi's method (4). There is an average difference of 25 milligrams per 100 cc. of glucose between the true blood sugar as determined by this method, and the apparent blood sugar, as estimated by the Folin-Wu-Reiner method.

TABLE 25
Normal Range of Blood Sugar as Determined by Various Micromethods

Method	Range, mg./100 cc.	Blood sugar designation
Hagedorn-Jensen (2).......	95–135	Apparent
Boyd (3).................	65–80*	True
Folin (5).................	70–110	Apparent
Folin-Wu-Reiner (6).......	80–120	Apparent
Somogyi (4)..............	60–100	True

* Personal communication from Dr. J. D. Boyd.

FOLIN'S MICROMETHOD (5)

PROCEDURES

Reagents.

(*1*) Dilute tungstic acid solution. Transfer 20 cc. of 10 per cent sodium tungstate solution to a volumetric one-liter flask; dilute to a volume of 800 cc.; add, with shaking, 20 cc. of $^2/_3$ *N* sulfuric acid and dilute to volume.

(*2*) Sodium cyanide–carbonate solution. Transfer 8 Gm. anhydrous sodium carbonate to a 500 cc. volumetric flask; add 40–50 cc. water and shake, to promote solution; with a cylinder, add 150 cc. freshly prepared 1 per cent sodium cyanide solution, dilute to volume, and mix.

(*3*) Potassium ferricyanide solution. Dissolve 1 Gm. potassium ferricyanide in distilled water and dilute to a volume of 500 cc. Place the major part of this solution in a brown bottle and keep in the dark. Keep the portion intended for daily use also in a brown bottle.

(*4*) Ferric iron solution. Fill a one-liter cylinder with water, and push into the cylinder a circular piece of window screening large enough to form a bowl well below the surface of the water. On the screen suspend 20 Gm. soluble ghatti gum, cover the cylinder, and let stand for 18 hours. Remove the screen and strain the liquid through a double layer of clean towel or a filter. Transfer 5 Gm. ferric sulfate, $Fe_2(SO_4)_3.9H_2O$, to another, smaller flask, add 75 cc. of 85 per cent phosphoric acid and 100 cc. of water, and heat until the sulfate has dissolved. Cool and transfer the solution to the ghatti gum extract. To the mixture add, a little at a time, about 15 cc. of 1 per cent potassium permanganate solution to destroy certain reducing materials present in the ghatti gum. The slight turbidity of the solution disappears completely if it is kept at 37 C. for a few days. Do not shake vigorously.

(*5*) Standard glucose solutions. Dissolve 2 Gm. benzoic acid in about 500 cc. hot distilled water in a one-liter flask. Weigh out 2 Gm. glucose, and rinse it by means of the warm benzoic acid solution into a volumetric one-liter flask. Add water to about 900 cc., cool to room temperature, dilute to volume, mix, and transfer to a clean, glass-stoppered bottle. The solution, which contains 2 mg. glucose per cubic centimeter, is the stock solution.

Dilute working standard, containing 0.01 mg. glucose per cubic centimeter: Transfer 0.5 Gm. benzoic acid to a 2-liter volumetric flask, add about 1,500 cc. distilled water and 10 cc. of the stock solution, and shake until the benzoic acid has dissolved. Dilute to volume, mix, and transfer to a glass-stoppered bottle. In order not to contaminate this solution by constant uncorking, transfer a small part for daily use to a separate container.

Technic. 10 cc. dilute tungstic acid solution are measured into a 15 cc. centrifuge tube. 0.1 cc. whole blood is collected with an 0.1 blood micropipet and transferred into the centrifuge tube. The pipet is rinsed with the mixture. The contents of the tube are stirred well, and centrifuged for 3–5 minutes. 4 cc. of the water-clear supernatant fluid are transferred to a test tube graduated at 25 cc. (Folin-Wu sugar tube). Into a similar tube are measured 4 cc. of the dilute working standard glucose solution. To each tube are added 1 cc. of the potassium ferricyanide solution and 1 cc. of the sodium cyanide–carbonate solution, and both tubes are heated in boiling water for 8 minutes, then cooled in running water for $^1/_2$ minute. 3 cc. of the ferric iron solution are run down the wall of each tube, and the contents are thoroughly mixed. The tubes are allowed to stand for $^1/_2$ minute; then water is added almost to the 25 cc. mark. The surface of the mixture in the tubes is apt to be obscured by the presence of a little foam; this can be destroyed by adding a few drops of alcohol, after which dilution to the 25 cc. mark exactly is completed and mixing is repeated. The green-colored solutions are now ready for reading on the ordinary colorimeter.

Calculation.

$$(St/R) \times 100 = \text{milligrams blood sugar per 100 cc.}$$

where St is reading of the standard glucose solution and R is reading of unknown (blood) solution.

REINER'S MICROMODIFICATION (6) OF FOLIN-WU METHOD (7)

This modification will be found particularly convenient by those accustomed to the universally used Folin-Wu method for determination of blood sugar in adults. The same reagents and standards are used in both methods, and no additional apparatus are needed.

PROCEDURE

Apparatus. "Sugar tubes" calibrated at 12.5 and 25 cc., with a constriction above a 4 cc. bulb at the bottom.

Reagents.

(*1*) Folin's copper reagent. Dissolve 40 Gm. anhydrous sodium carbonate in 400 cc. of water and transfer to a one-liter volumetric flask. Add 7.5 Gm. tartaric acid and 4.5 Gm. crystalline copper sulfate, mix the contents of the flask until all particles have dissolved, and make up to volume with water. Filter the solution if it is not clear.

(*2*) Phosphate–molybdate mixture. Measure 35 Gm. molybdic acid, 5 Gm. sodium tungstate, 200 cc. 10 per cent sodium hydroxide, and 200 cc. water into a one-liter beaker. Boil the mixture for 20–40 minutes, cool overnight, and filter into a 500 cc. volumetric flask; wash the residue on the filter with a small amount of water; add 125 cc. 85 per cent phosphoric acid and water to volume.

(*3*) 10 per cent sodium tungstate solution in water.

(*4*) $^1/_{12}$ *N* sulfuric acid.

(*5*) Stock sugar solutions. Prepare 1 per cent glucose solution (stock solution). Add 250 cc. water to 5 cc. of stock solution (dilute stock solution). Both solutions should be kept under toluene. The dilute stock solution, which contains 20 mg. glucose per hundred cubic centimeters, keeps for a week.

Technic. From finger tip, toe, or heel, 0.2 cc. of whole blood is collected, transferred into a small tube containing 1.6 cc. of $^1/_{12}$ *N* sulfuric acid, and the pipet rinsed with the mixture. 0.2 cc. of 10 per cent sodium tungstate is added, and the tube centrifuged for 5 minutes. 1 cc. of the supernatant fluid is then transferred into a 25 cc. Folin-Wu sugar tube, 1 cc. of water and 2 cc. of the copper reagent are added, and the tube is placed in a boiling water bath for 7 minutes and then cooled in running water. 2 cc. of the phosphate–molybdate mixture are added, and water to the 12.5 cc. mark. The solution is mixed thoroughly and is then ready to be read by visual colorimetry against one of the standards prepared at the same time. Readings should be made within 10 minutes of adding the molybdate.

Two standard sugar solutions are prepared and analyzed in sugar tubes according to the Folin-Wu macromethod: (*1*) light standard, containing 1 cc. of the dilute stock glucose solution and 1 cc. of water, and (*2*) dark standard, containing 2 cc. of the dilute stock glucose solution. To each of the 2 tubes, 2 cc. of copper reagent are added. The tubes are boiled, cooled, and 2 cc. of the phosphate–molybdate mixture are added and water to the 25 cc. mark.

Calculation.

$$\frac{\text{Standard}}{\text{Unknown}} \times \text{mg. glucose in standard} \times \frac{100}{0.2} = \text{mg. glucose per 100 cc.}$$

For the normal range of blood sugar, see Table 25.

TOLERANCE TESTS

ONE-DOSE ORAL GLUCOSE TOLERANCE TEST

The reaction to a standard dose of glucose is ascertained by repeated analyses of blood and urine for glucose. A blood glucose tolerance curve is obtained by determining the glucose level in the blood at 30 to 60 minute intervals after ingestion of the test dose. A normal, a high prolonged, and a low rise of the blood sugar curve indicate, respectively, normal, decreased, or increased tolerance.

Glucose tolerance, as shown by the shape of the curves obtained by oral tests, is the result of at least two component functions, namely, absorption and utilization. Conclusions as to intermediary disturbances may be drawn only if it may be safely assumed that the results are not materially influenced by an abnormal rate of intestinal absorption. The test is of particular value in revealing disorders of carbohydrate metabolism in the absence of hyperglycemia and glycosuria, and in differentiating conditions associated with hyperglycemia and/or melituria.

PEDIATRIC CONSIDERATIONS

All the available data point to the fact that the capacity to utilize carbohydrates is considerably higher in children than in adults. Furthermore, the renal threshold for sugar, also, is higher in children. When equal amounts of dextrose per kilogram of body weight are ingested by adults and children, adults show a higher optimal rise in blood sugar than children; and relatively larger quantities of sugar are required to evoke glycosuria in infants and children (Table 26). From the age of 6 years on, the child's tolerance for sugar gradually decreases to the average adult capacity. The hyperglycemic response of children to standard test doses of glucose referred to kilogram of body weight, therefore, varies with age. The dose commonly used is 1.75 Gm. per kilogram of body weight, with a minimum dose of 10 Gm. and a maximum dose of 50 Gm., so that children weighing less than 6 Kg. (or under 6 months of age) receive relatively larger doses. The average response as observed in normal children of similar body weight serves as the basis for interpreting the test results. It is inadvisable to consider age, beyond the limitations given above, in figuring the standard dose per kilogram of body weight.

TABLE 26

Minimal Doses of Various Sugars (in Gm./Kg. Body Weight) Required to Produce Alimentary Glycosuria

Age	Glucose	Saccharose	Lactose	Levulose	Galactose
Infants	5.0	3.1–3.5	3.1–3.6	2.6	2.2–4.0
Adults	2.0–2.5	2.0–2.8	1.0–1.7	1.5	0.6

After Knauer (8).

Formerly, the standard dose for adults was also 1.75 Gm. per kilogram of body weight, but the practice now is to give 1 Gm. per kilogram (9).

PROCEDURE

Provided clinical conditions will permit it, the child should be on a diet normal for its age for 4 to 5 days before the test.

The test should be started in the morning, after a fasting period of at least 8 hours. The standard dose of glucose (see above) is given in 20 per cent aqueous solution made palatable with a few drops of lemon juice. Administration by gavage is necessary only when the child refuses to drink the solution. Capillary blood is taken from the finger, toe, or heel, before and ½, 1, 1½, 2, and 3 hours after ingestion of the test dose. Urine is collected prior to the test (control urine), and 1 and 2 hours after ingestion of the test dose. Infants and small children have to be catheterized. Because the significance of alimentary glycosuria is frequently disregarded, urinalysis in young children is too often omitted.

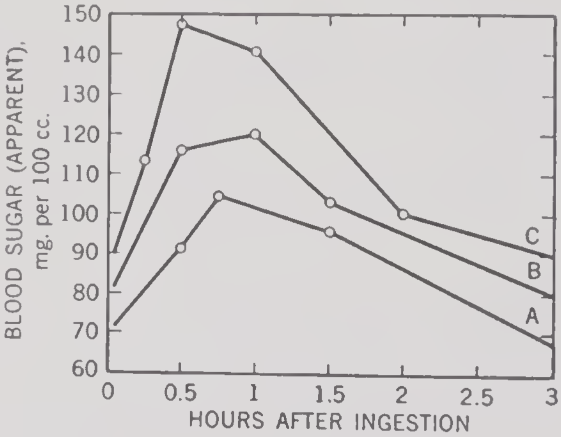
Blood sugar is determined by one of the micromethods already described (page 99).

INTERPRETATION

One of the factors determining the blood sugar curve is the child's diet during the week preceding the test. As compared to a regular diet for the age, a high carbohydrate diet lowers the hyperglycemic response, whereas high fat or ketogenic diets have the opposite effect. A low caloric diet also reduces carbohydrate tolerance (10). Infants fed cow's milk, which is high in phosphate, show lower glycemic reactions than those fed mother's milk, which is low in phosphate (11). These facts should be borne in mind when the clinical significance of sugar tolerance curves are being evaluated.

The response of normal children varies greatly, even in the same child. There is no "normal" blood sugar tolerance curve; it is rather a certain range of blood sugar responses which may be considered typical of a normal tolerance. All the values and curves given below are merely averages of such typical blood sugar changes, and they are valid only provided the standard procedure of the test has been adhered to. When capillary blood is used, the alimentary hyperglycemia is higher by 20 to 40 mg. per hundred cubic centimeters than when venous blood is tested (12a).

Fig. 8. Oral glucose tolerance test, using capillary blood. Normal responses in children of different ages to standard test doses. Based partially on data of Greenwald and Pennel (13) and McLean and Sullivan (12b). A: Newborn. B: From 1 month through 5 years of age. C: Over 5 years.



The average normal response (12b) of a child to the oral glucose tolerance test is a rise in the blood sugar by 30 to 40 mg. per hundred cubic centimeters, reaching the peak 30 to 45 minutes after ingestion of the test dose. Only a small percentage of children reach values of 140 to 150 mg. per hundred cubic centimeters. The return to the fasting level occurs more or less uniformly within 2 to 2½ hours. Glycosuria is absent throughout the period of observation. The newborn show practically the same response (13), but the fasting point, as a result of the physiologic hypoglycemia of the newborn, is generally lower than in older infants. Figure 8 shows glucose tolerance curves of normal children at varying ages.

Abnormal responses (Fig. 9) consist in (*1*) a high blood sugar curve, i.e., elevation of the blood sugar above 150 mg. per hundred cubic centimeters; (*2*) prolonged blood sugar curve, i.e., failure of the blood sugar to return to the starting point within 3 hours; (*3*) high prolonged curve, i.e., a combination of the first two; and (*4*) a flat curve, i.e., a rise of not more than 30 mg. per hundred cubic centimeters above the fasting level.

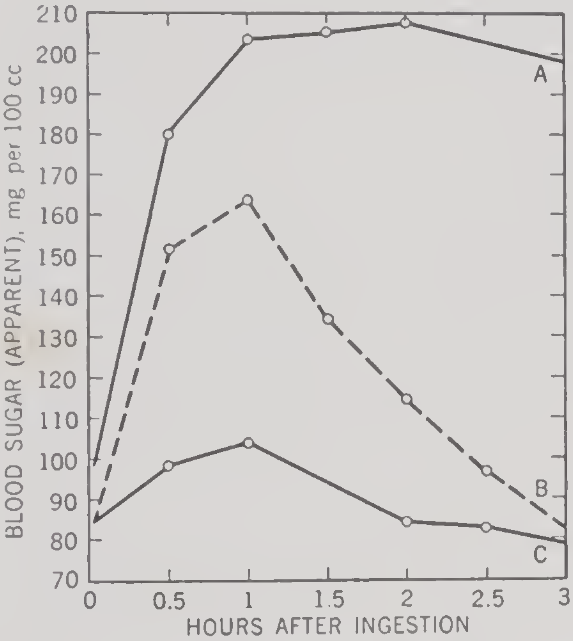


Fig. 9. Oral glucose tolerance test. Three typical instances of abnormal responses in children to standard test doses. A: High prolonged curve in a 6 year old diabetic child under adequate control. B: High curve in a healthy 10 year old child. C: Flat curve in a 1 year old cretin.

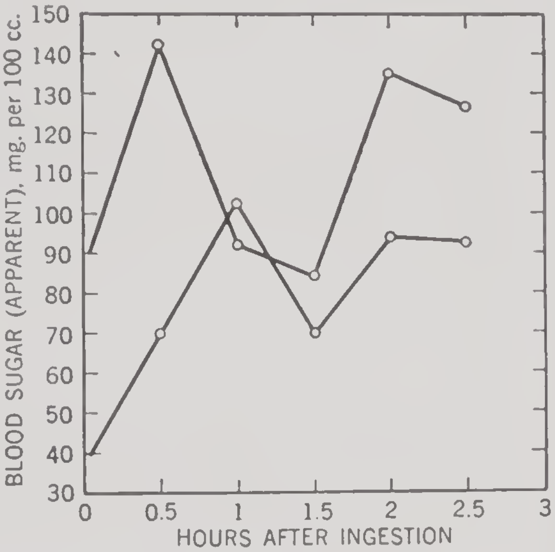


Fig. 10. Oral glucose tolerance test, using capillary blood. "Diphasic" high prolonged blood sugar curves in a 6 year old boy with glycogen storage disease. From van Creveld (15).

High blood sugar curves have no pathologic significance. Prolonged curves, of normal or abnormal height, are always a sign of lowered tolerance. When combined with glycosuria, they represent the typical response of diabetic children, irrespective of the actual fasting blood sugar level (Fig. 9). However, not every high prolonged curve is indicative of diabetes. This type of curve is found in various hepatic disorders, with or without jaundice, and is widely considered as a sign of impaired hepatic function (page 98). The same high prolonged response has been observed in children in various diseases (septicemia, scarlet fever, pneumonia, tuberculous meningitis, acute nutritional disturbances), when no liver involvement was apparent clinically. But in these cases the urine is ordinarily free of sugar. High prolonged curves have also been

TABLE 27

Typical Responses to Various Tests of Carbohydrate Metabolism in Hypoglycemic Conditions in Childhood

	Responses common in			
Test	hyper-insulinism	hepatic disorders	glycogen storage disease	fatty infiltration of liver
Fasting blood sugar	Low	Low	Low	Low
Pre-existing ketosis	Absent	Absent	Often present	Absent
Pre-existing hypoglycemic symptoms	Present	Absent	Absent or present	Absent
Glucose tolerance (oral test)	Increased	Decreased	Decreased	Decreased
Blood sugar response	Flat curve	High prolonged curve	High prolonged "biphasic" curve	High prolonged curve
Glucosuria	Absent	Absent	Absent	Absent
Adrenalin effect	Normal; occasionally absent	Slight or normal	Abnormal	Abnormal
Blood sugar response	Marked rise; seldom absent	Slight to marked rise	Absent; occasional slight rise	Absent or slight rise
Ketosuria	Absent	Absent	Present	Absent
Insulin tolerance	Hypersensitivity	Hypersensitivity	Normal; occasionally low	?
Blood sugar response	Steep fall	Moderate fall	Moderate fall	?
Shock symptoms	Present	Absent	Absent or present	?

found in chronic hypoglycemic conditions of hepatogenic origin. These are glycogen storage disease, which shows a peculiar biphasic rise (Fig. 10), and fatty infiltration of the liver with aglycogenesis (14). In neither group has glycosuria been observed during or following the test (Table 27).

Impaired tolerance, manifested by high prolonged curves and glycosuria, develops in children conditioned by a low carbohydrate intake, a condition known as starvation diabetes.

Flat blood sugar curves prevail among patients with encephalitis and hypothyroidism (Fig. 9). They are pathognomonic for the celiac syndrome, in which they are the result of impaired intestinal absorption (16,17), and for hyperinsulinism (Table 27).

A normal response of the blood sugar, coupled with alimentary glycosuria, is the typical finding in patients with renal diabetes.

TWO-DOSE ORAL GLUCOSE TOLERANCE TEST

The Exton-Rose procedure (18) makes use of the fact that in normal individuals repeated ingestion of dextrose leads to ever-increasing sugar tolerance (Staub-Traugott effect), so that after the second or third ingestion of dextrose the alimentary hyperglycemia is less marked and is sometimes replaced by hypoglycemic reactions.

This test is useful in revealing disorders of carbohydrate metabolism, particularly those caused by hepatic disease, when the one-dose test is unsuccessful.

PROCEDURE

Preparation of the child, sugar dosage, collection of blood, and microdetermination of the blood sugar are essentially the same as for the one-dose test, except that the total amount of dextrose is divided into two equal doses. The first dose is given at the beginning of the test, the second 30 minutes later. Samples of blood and urine are collected before administration of the first dose, immediately before administration of the second dose, and 30 minutes after the second dose.

INTERPRETATION

Normal infants and children, according to Cooperstock and Galloway (19a), show a moderate hyperglycemia 30 minutes after the ingestion of the first dose; 30 minutes after the second dose (60 minute sample) the blood sugar declines sharply in the majority of cases, sometimes dropping below the fasting level. A different reaction, however, is observed in a small percentage of normal children, especially in infants. Instead of the "relative" hypoglycemia, a second rise of the blood sugar occurs following the second dose. This rise never exceeds the fasting level by more than 80 mg. per hundred cubic centimeters, and the 30 minute level rarely by more than 10 mg. per hundred cubic centimeters (Fig. 11, A–C).

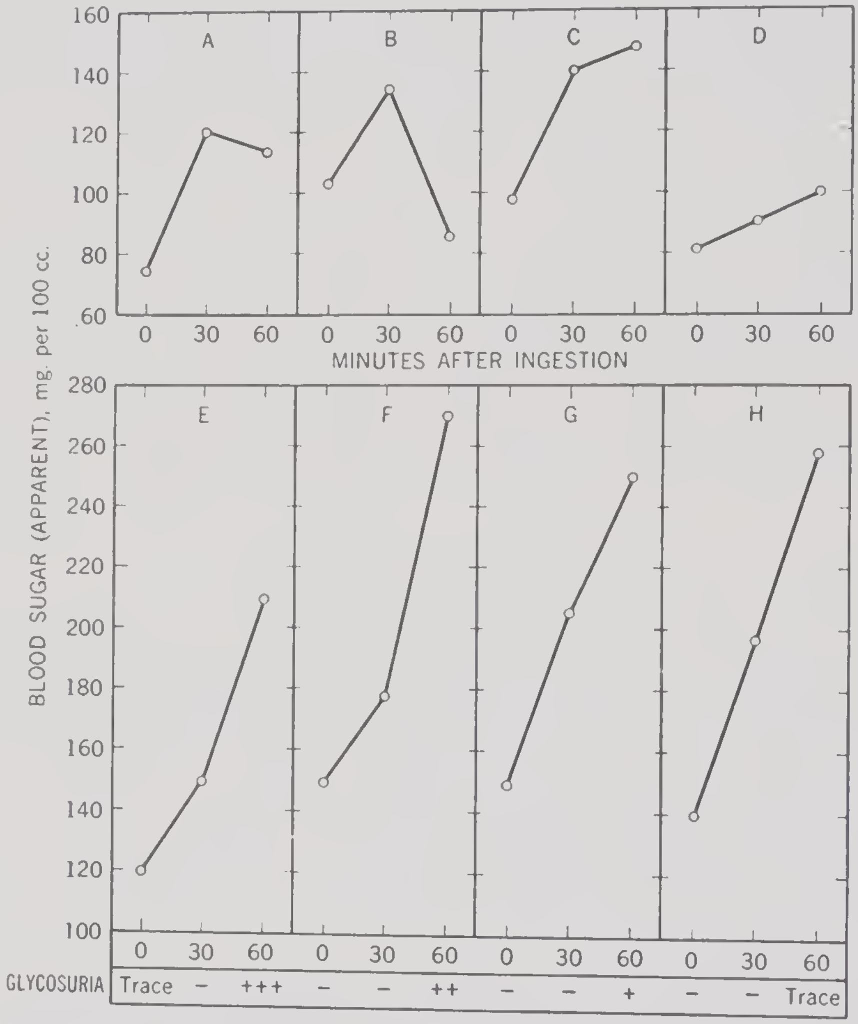


Fig. 11. One-hour, two-dose oral tolerance test, using capillary blood. First test dose ingested at 0, second dose at 30 minutes. A–C: The three types of normal response, obtained in children aged 1 month, 12 years and 7 years, respectively. D: Response of a 16 month old cretin. E–G: Responses of diabetic children, 11, 8, and 2 years old, respectively. The diabetes varied in severity and duration, but in all three was under satisfactory control. H: Response of an 8 year old hyperthyroid child. From Cooperstock and Galloway (19a).

The Staub-Traugott effect is absent in premature infants during the first weeks of life and in many full term infants under 3 months of age (19b). No glycosuria has been found with any of these types of normal response.

In children with a decreased tolerance for sugar, the second test dose provokes a second sharp rise of the blood sugar, reaching a level about twice as high as the fasting value and exceeding the 30 minute level by 30 mg. or more per hundred cubic centimeters. Such curves are typical of the response of diabetic children (Fig. 11, D–F). In these patients, glycosuria is found regularly in the 60 minute specimen.

The diabetic type of curve, but without concomitant glycosuria, may also occur in hyperthyroidism (Fig. 11 G).

Increased tolerance is found in hypothyroidism, hyperinsulinism, and when there is interference with intestinal absorption of the test dose (Fig. 11 F). The 30 minute and 60 minute peaks are almost absent.

INTRAVENOUS GLUCOSE TOLERANCE TEST

According to Crawford's procedure (20), the child is prepared as for the oral test. The standard dose for infants and children is 0.5 Gm. glucose per kilogram of body weight, injected as a 20 per cent solution in 0.9 per cent sodium chloride, at a rate of 45 seconds for each 20 cc. Samples of capillary blood are taken 2 minutes after injection, and again 15, 30, 45, 60, and 90 minutes after injection. The blood sugar determination is carried out by one of the methods already described.

Urine samples are taken before the injection is given, and when the last specimen of blood has been withdrawn, and both samples are examined for sugar.

INTERPRETATION

In normal children the blood sugar rises to its maximum immediately after the injection is completed. Thereafter a rapid decline sets in and within 45 to 75 minutes the level returns to the fasting value (Fig. 12). Blood withdrawn thereafter frequently shows subnormal sugar concentration.

The response is considered abnormal when the blood sugar does not decline to 100 mg. per hundred cubic centimeters within 60 minutes in children under 4 years old, or within 75 minutes in children over 4 years. It is also abnormal if the curve shows an unusually steep fall to normal or subnormal values, so that the 30 minute sample contains less than 100 mg. per hundred cubic centimeters.

No definite conclusions can be drawn from a child's abnormal response to the intravenous test, and the variable results of tests repeated at short intervals on the same child (21) lessen the diagnostic value of the intravenous method. Nevertheless, while the result does not necessarily reflect the ability of the liver to store glycogen, abnormal curves do suggest some functional impairment of liver or pancreas.

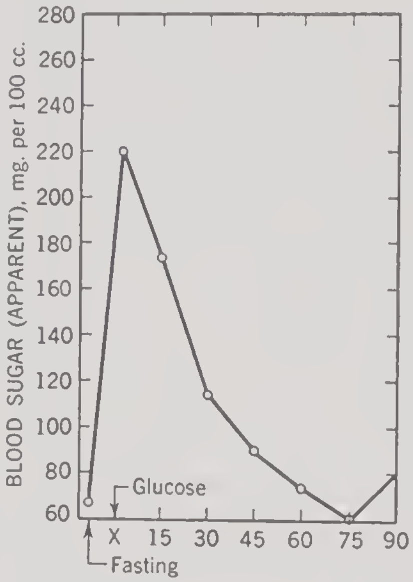


Fig. 12. Intravenous glucose tolerance test. Normal response. After Pachman (21).

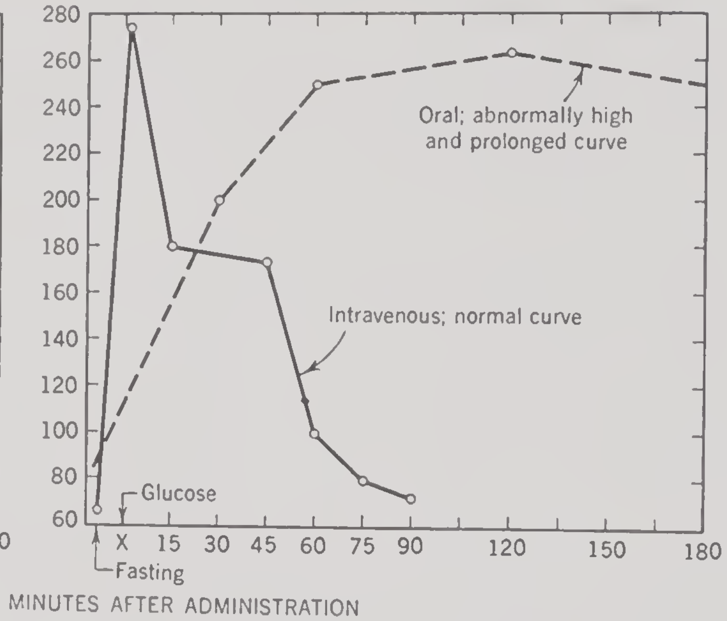


Fig. 13. Oral and intravenous glucose tolerance tests in a 5 year old boy with primary carcinoma of the liver. From Wentz and Kato (22).

Oral and intravenous tests, performed on consecutive days, do not invariably show similar results. In this connection, the observations that have been made on children with severe hepatic disorders, such as cirrhosis or carcinoma (22), of the liver (Fig. 13), portal obstruction, prolonged catarrhal jaundice (21), Banti's syndrome, and the Smith-Howard-Wallgreen syndrome are of clinical interest. The curve obtained with the oral test in the above conditions is of the high prolonged type, while the intravenous test may reveal a normal curve. No satisfactory explanation has so far been found for this divergence. The general experience has been that when the intravenous test shows a lowered tolerance, the tolerance to orally administered glucose also is decreased.

LEVULOSE TOLERANCE TEST

The test measures the ability of the liver to convert ingested levulose (fructose) to dextrose and glycogen. Normally, only small amounts of levulose pass into the general circulation after ingestion. An abnormal rise in blood levulose levels is attributed to a failure of the liver to dispose of the ingested levulose at a normal rate.

The older procedure (23–27) was based on the assumption that a rise in blood levulose would result in a corresponding increase in total blood sugar. Consequently, the levulose level was deduced from variations in the total amount of reducing substances. This concept has been shown to be fallacious, and it is now well established that in many instances the blood glucose also reacts to the ingestion of levulose, thus masking the changes in total blood sugar which should be ascribed to the levulose.

The new method (28) estimates blood levulose directly, and so eliminates the errors. Following ingestion of a standard dose of levulose, a blood levulose curve is obtained, the form or course of the curve demonstrating the degree of tolerance. The additional determination of the total blood sugar (total reducing substances) is of value in establishing the relationship between blood glucose and blood levulose and their response to the levulose loading test in diabetic subjects.

Small children and infants can easily be tested, now that a micromethod is available, and the test constitutes an important aid in assessing the functional capacity of the liver.

PROCEDURE

The method is that of Thompson and Wilkinson (29). The child should be on a normal diet for its age prior to the test, which is carried out after an overnight fast. The standard dose of levulose for children is 1 Gm. per kilogram of body weight. This is given by mouth, as a freshly prepared, 10 to 20 per cent aqueous solution. 0.2 cc. of capillary blood is taken before the test, and ½, 1 and 2 hours after ingestion of the test dose. The blood levulose curve is obtained by estimating the levulose content of each sample (see below). If the glucose curve is to be determined at the same time, two specimens are taken each time. The glucose values are obtained by subtracting the levulose values from the total sugar values of the second set of specimens.

INTERPRETATION

Normally, there is only a slight rise in blood levulose levels, the maximum increase averaging 7.1 mg. per hundred cubic centimeters and occurring about an hour after ingestion of the test dose.

The highest rise that has been observed in healthy children is 14 mg. per hundred cubic centimeters. The return to fasting level is completed within 2 hours (Fig. 14).

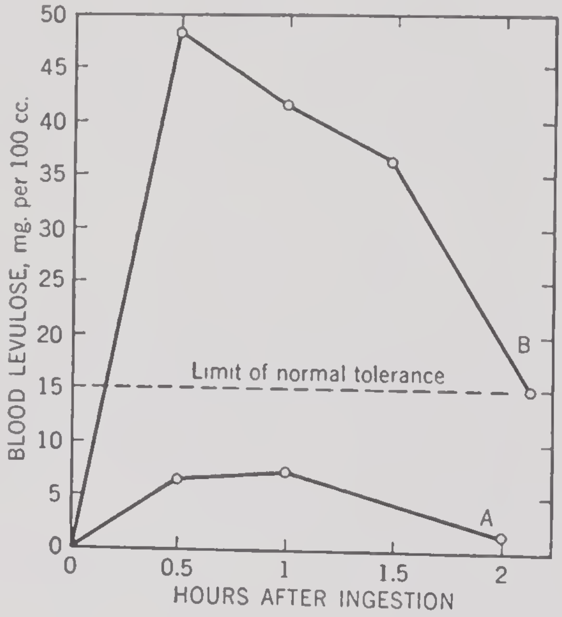


Fig. 14. Levulose tolerance test, using capillary blood. Responses in children to standard test doses. A: Average normal curve. According to Thompson and Wilkinson (29). B: Abnormally high curve, indicating low tolerance, in a 7 year old child with levulosuria. From Oster (30).

A rise exceeding 15 mg. is considered abnormal. Sometimes, an abnormally high increase is combined with failure to return to the fasting level within 2 hours. All high or prolonged curves are a sign of impaired tolerance, while an unusually low rise is indicative of a high tolerance (Fig. 14).

The following summarizes the clinical significance of results of the levulose tolerance test.

(*1*) A normal levulose tolerance does not rule out the presence of liver damage, for the liver has an enormous functional reserve and deficient function may not become manifest until considerable destruction has occurred.

(*2*) Decreased levulose tolerance is an indication of hepatic insufficiency, resulting from extensive damage to the liver parenchyma. In cases of prolonged jaundice the test does not provide an unequivocal basis for differentiation between mechanical obstruction and toxic or infectious hepatitis. Although extremely high levulose curves support the diagnosis of primary hepatogenous jaundice, such curves also occur in prolonged obstructive jaundice with secondary damage to the liver (page 27).

(*3*) A markedly low tolerance is one of the principal signs of essential fructosuria, a congenital disorder of carbohydrate metabolism. Recently, Sachs, Sternfeld, and Kraus (31) reviewed the subject of levulosuria in children, and further details may be found in their report.

MICROMETHOD FOR ESTIMATION OF BLOOD LEVULOSE

The method is that of Patterson (32) and Herbert (33), as modified by Thompson and Wilkinson (29).

Reagents.

(*1*) 2.25 per cent zinc sulfate solution ($ZnSO_4.7\,H_2O$).

(*2*) 0.1 *N* sodium hydroxide.

(*3*) Acid–alcohol–diphenylamine reagent. 70 parts alcohol, 50 parts concentrated hydrochloric acid, and 1 part 20 per cent solution of diphenylamine in alcohol. In a brown bottle the diphenylamine solution will keep for a month. Prepare the reagent immediately before use.

(*4*) Standard levulose solution. 1 per cent levulose solution in saturated benzoic acid.

(*5*) Dilute standard levulose solution. Dilute 0.5 cc. standard solution to 400 cc. shortly before use. 1 cc. dilute standard contains 0.0125 mg. levulose.

Technic. From the finger tip, toe, or heel 0.2 cc. of blood is drawn into a 0.2 cc. micropipet and the blood is transferred directly into a test tube containing 2 cc. zinc sulfate solution. 2 cc. of 0.1 *N* sodium hydroxide are added, the contents of the tube are mixed, the tube is heated in a boiling water bath for 3 minutes, and is then cooled. The fluid is filtered through a 5.5 cm. filter paper; 2 cc. of the protein-free filtrate are pipetted into a 6 × ⅝ in. pyrex test tube graduated at 10 cc., and 6 cc. of diphenylamine reagent are added. Into a second tube are measured 2 cc. of dilute levulose solution and 6 cc. of diphenylamine reagent. Both tubes are heated in a boiling water bath for exactly 15 minutes, cooled rapidly in running water, the contents made up to 10 cc. with alcohol, and compared in a colorimeter.

Calculation.

$$\frac{\text{Standard}}{\text{Unknown}} \times 0.025 \times \frac{100}{0.095} = \text{mg. levulose per 100 cc.}$$

GALACTOSE TOLERANCE TEST

When ingested galactose reaches the liver it is converted into glucose and stored as glycogen. This transformation is incomplete if there is hepatocellular damage, and considerable amounts of galactose pass through the liver into the general circulation and are excreted in the urine. Examination of blood and urine, therefore, reveals how much of the ingested galactose escapes the liver barrier. This postabsorptive concentration of galactose in blood and urine is also affected by changes in the rates of intestinal absorption and of renal excretion.

The test, as originally devised by Bauer (34), consisted of oral administration of a dose of 40 Gm. of galactose, and subsequent urinalysis for galactose. The newer procedure (35) follows the galactose level in the blood for 2 to 3 hours after oral administration of a standard dose of galactose. An abnormally high rise in the galactose level is a sign of impaired tolerance. If it seems desirable, both the blood curve and the urinary excretion of galactose may be ascertained.

Basset, Althausen, and Coltrin (36) have recently described an intravenous galactose tolerance test. When the test dose is administered in this manner, the functional capacity of the liver is primarily revealed without being masked by variations in intestinal absorption.

PEDIATRIC CONSIDERATIONS

Children of all ages have been tested for galactose tolerance, although not nearly as frequently as adults. For practical reasons, oral administration of the test dose is preferred. Estimation of galactose in the blood may be done by a micromethod which requires only 0.2 cc. of whole blood. Although the proper test doses for children of various ages have been investigated, they are not yet standardized. The effectiveness of the selected test dose, therefore, should be checked by performing a control test with the same dose in a healthy child of approximately the same age and weight.

In children, as in adults, determination of galactose tolerance is valuable as a liver function test, as an aid in diagnosing hyperthyroidism, and as a means of recognizing hepatogenic abnormalities in carbohydrate metabolism. There is no evidence that substitution of the disaccharide lactose for galactose as a test substance (37) increases the test's significance or accuracy.

PROCEDURES

Test Dose. The test dose, in the form of a 10 per cent solution flavored with lemon juice, is administered in the morning, on a fasting stomach. For infants and children up to the age of 3, the commonly recommended dosage is 1.75 Gm. per kilogram of body weight (38,39). For all other age groups, dosages calculated on the basis of 1.75 Gm. per kilogram of body weight are considered unnecessarily large, although the doses actually proposed are not uniform. Thus, children between the ages of 2 and 5 years have been tested with doses of 15 to 20 Gm. (38); and for children between the ages of 5 and 8, the dosage used ranges between 20 and 40 Gm. Children over 8 years old are given the same dose as adults, namely, 40 Gm. dissolved in 400 cc. of water and ingested over a period of 10 to 15 minutes (40).

Urine Collection and Analysis. A urine specimen (fasting) is obtained prior to ingestion of the galactose; thereafter, urine is collected for a period of 5 hours, the individual specimens being pooled and the total amount recorded. The galactose is determined by titrating an aliquot of the pooled urine against Benedict's quantitative copper solution, 1 cc. of which is reduced by 0.0025 Gm. of galactose.

If the fasting specimen gives a positive reduction result, indicating the presence of glucose, it must be removed by treating the pooled specimen with yeast. This is done by adding 7.7 parts of a 10 per cent yeast suspension (see below) to 1 part of urine, incubating the mixture at 37 C. for 45 minutes and filtering. The nonfermentable sugar, i.e., the galactose, is estimated in an aliquot of the filtrate.

Blood Collection and Analysis. The procedure described is that of Althausen, Lockhart, and Soley (41). Blood is taken from the pricked finger before and 30, 60, 90, and 120 minutes after administration of the galactose. The concentration of galactose in each sample is determined as glucose, after the blood has been cleared of its glucose by fermentation with yeast.

The 10 per cent yeast suspension is prepared by suspending a weighed amount of fresh commercial yeast in 5 parts of water. After centrifugation, the supernatant fluid is decanted, the yeast is resuspended in water, and centrifuged. This process is repeated 6 of 7 times, until the supernatant fluid is clear and colorless and gives no reduction reaction. The yeast is then suspended in 10 parts of water. In the refrigerator the suspension will keep for 2 weeks.

Depending on personal preference, one of the described methods (page 99) or any other method of determining blood glucose may be used for estimating blood galactose. Provision must be made that, after addition of yeast and precipitation of proteins, the aliquots of protein-free extracts measured out for analysis are always equivalent in blood content to that quantity of extract specified in the original method. The following procedures may serve as examples.

By *Folin's micromethod*, 0.1 cc. of whole capillary blood is collected with a pipet and transferred directly into a 15 cc. centrifuge tube containing 2.6 cc. of water. The pipet is rinsed with the mixture, and 1 cc. of the 10 per cent yeast suspension is added. The contents of the tube are mixed thoroughly, and allowed to stand for 5 minutes; 0.2 cc. of $^2/_3$ N sulfuric acid, 6 cc. of water, and 0.2 cc. of 10 per cent sodium tungstate are then added, and the mixture is stirred and centrifuged for 5 minutes. 4 cc. of the water-clear supernatant fluid are analyzed, as outlined on page 101. To obtain the true value for galactose, 24 per cent must be added to the glucose value.

By *Hagedorn and Jensen's method* (2), 0.2 cc. of whole capillary blood is transferred to 2.3 cc. of distilled water and the pipet is rinsed with the mixture. 1 cc. of the 10 per cent yeast suspension is added, the contents of the tube are mixed, and after 5 minutes 0.5 cc. of tungstic acid solution is added. This solution is prepared freshly by mixing equal volumes of 10 per cent sodium tungstate solution and $^2/_3$ N sulfuric acid. The contents are mixed again, allowed to stand for a few minutes, and the mixture is centrifuged at high speed; 1 cc. of the clear supernatant fluid is used for titration. The result is converted into a galactose value by adding 24 per cent to the glucose value.

In the Folin-Wu macromethod (7), 2 cc. of oxalated blood, 14 cc. of a 20 per cent yeast suspension, 2 cc. of 10 per cent sodium tungstate , and 2 cc. of $^2/_3$ N sulfuric acid are the quantities to be used; 2 cc. portions of the clear filtrate are used for determination. Galactose values are obtained by conversion, as under the Folin micromethod.

INTERPRETATION

Urinary Excretion of Galactose. A total excretion of 2 Gm. or less of galactose in the pooled urine specimen, after ingestion of

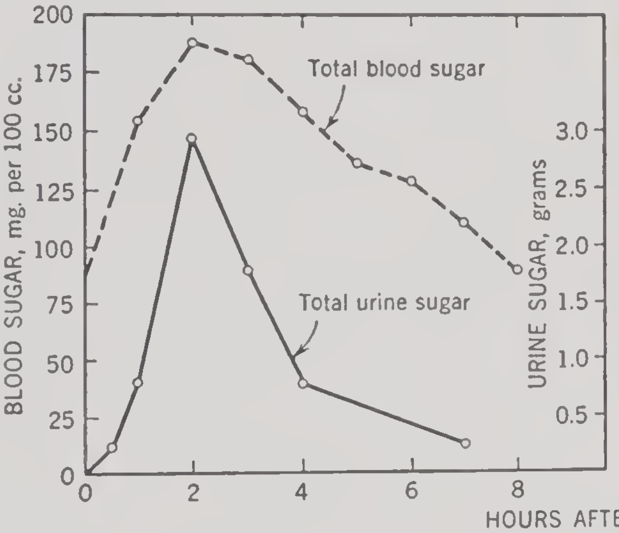


Fig. 15. Galactose tolerance test. Conspicuous intolerance exhibited by a 6 month old infant with chronic galactosemia. Test dose, 1.75 Gm. galactose per kilogram of body weight, administered orally. The values for total blood sugar and total urinary sugar represent values of nonfermentable sugar, calculated as dextrose. Total urinary sugar excreted during the 7 hour period amounted to 6.64 Gm. From Mason and Turner (38).

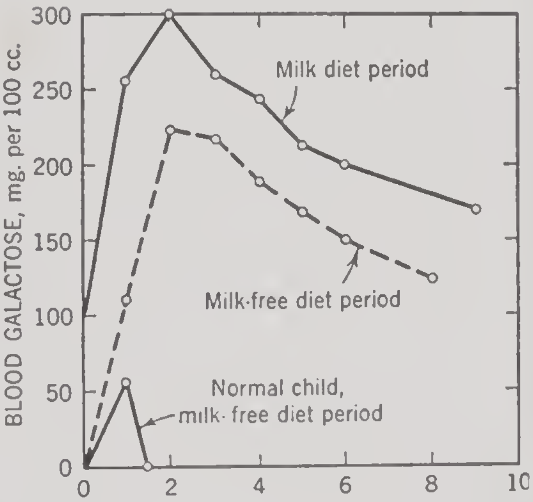


Fig. 16. Galactose tolerance test. Curves obtained in a 2 month old infant with chronic galactosemia and a normal infant of the same weight. Test dose, 1.75 Gm. galactose per kilogram body weight, administered orally. After Bruck and Rapoport (39).

40 Gm. of galactose, is considered normal. Healthy children, given smaller doses according to age, excrete less than 1 Gm., or none at all. In infants and small children, only an excessive galactosuria

(Fig. 15) should be considered as definitely abnormal, i.e., as evidence of lowered galactose tolerance.

Blood Galactose Curve. In normal children, as in adults, the galactose level reaches its peak within 30 to 60 minutes after ingestion of the optimal test dose. The concentration usually does not exceed 20 to 40 mg. per hundred cubic centimeters, and the return to the fasting level occurs before the end of the second hour (Fig. 16).

Abnormally high and prolonged curves are an indication of low tolerance. Values may rise to 100 mg. and more per hundred cubic centimeters during the first hour. The decline to the original level may occur within 2 to 3 hours (Fig. 17), or may be delayed for many hours (Fig. 15).

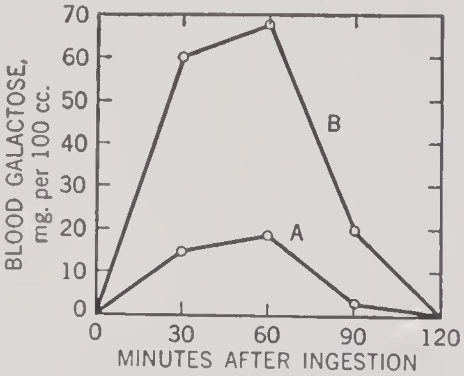


Fig. 17. Galactose tolerance test. Curves obtained in adults after oral administration of 40 Gm. galactose. A: Average normal. B: Abnormally high rise, indicating low tolerance; average of values obtained in 130 hyperthyroid patients. From Althausen, Lockhart, and Soley (41).

The "galactose index" (42) is the sum of blood galactose values, expressed in milligrams per hundred cubic centimeters, obtained 30, 60, 90, and 120 minutes after ingestion of galactose. This index cannot be used in children. In adults tested with the standard dose of 40 Gm., the average normal value of the index is 68, with an upper limit of the normal range at 160. Figures above 160 are a sign of definitely reduced tolerance.

The clinical significance of the test has been demonstrated for a number of childhood diseases. Reduced tolerance is a sign of impaired liver function (page 26), and in jaundice it supports the diagnosis of primary, hepatocellular pathology, i.e., parenchymatous jaundice (35). It is almost invariably found in hyperthyroidism (Fig. 17), so that the diagnostic value of the blood galactose curve in children with exophthalmic goiter is obvious. Decreased tolerance has also been found in pituitary dwarfism (40). Children with chronic galactosemia show a most conspicuous lack of galactose tolerance (38,43) (Figs. 15, 16). When these patients are on a milk diet they show a considerable degree of galactosemia even after a fast of 6 hours or longer. It may be better, therefore, to perform the tolerance test when the child is on a milk-free diet and has a fasting blood galactose level of zero (Fig. 16).

INSULIN TOLERANCE TEST

Sensitivity to insulin is judged by the degree of hypoglycemia which follows injection of a standard dose of insulin, the shape of the blood sugar curve indicating the extent of the reaction. Its mechanism is a rather complex one (44,45a), involving three factors: (*1*) the potency of the insulin-producing apparatus; (*2*) the effect of the contra-insular hormones of the pituitary and adrenal glands; and (*3*) the ability of the liver cells to mobilize glycogen. When the first factor only plays a part, "true" hyperinsulinism or hypoinsulinism results; when the last two factors are primarily concerned, the condition is characterized as "relative." The insulin tolerance curve provides no clue to the factor responsible for the altered sensitivity to insulin, and the diagnosis of true or relative hyperinsulinism or hypoinsulinism can only be made on the basis of clinical signs.

PEDIATRIC CONSIDERATIONS

Children respond to the insulin tolerance test in the same manner as adults. The standard test dose of insulin is adapted for children according to their weight, and micromethods are employed to determine the blood sugar curve. The test is diagnostically valuable in the study of hypoglycemic conditions, especially during periods when the blood sugar is at a normal level and the child is free of clinical symptoms. The test also serves as an aid in interpreting the causes of impaired sugar tolerance.

PROCEDURE

The method described is that of Daniels (46a). The child is tested while in a fasting state, preferably in the morning. The diet prior to the test should be the normal one for the age, insofar as the clinical condition permits. If both insulin and glucose tolerance tests are to be performed, the latter should always be done first.

with the insulin test following on the next day, if it can be conveniently managed.

The standard dose of 0.25 unit of insulin per kilogram of body weight is injected intracutaneously. Blood specimens are taken from the pricked finger or toe before and 20, 40, 60, 90, and 120 minutes after the injection, and blood sugar determinations are carried out by one of the methods described above.

INTERPRETATION

With the exception of the newborn, the average normal response is practically the same in infants and in children. The blood sugar drops steadily during the first 30 minutes following administration of the test dose. It reaches the lowest level between 30 and 60 minutes after injection, and the low level is maintained through the second hour. The difference between the fasting level and the lowest blood sugar level ranges between 15 and 25 mg. per hundred cubic centimeters.

If the decrease in the blood sugar level is less than 10 mg. per hundred cubic centimeters, or if the difference between fasting and minimal levels is greater than 35 mg. per hundred cubic centimeters, the response is considered abnormal.

Lack of response to the insulin test, as evidenced by a flat curve, is characteristic of diabetic coma accompanied by acidosis. An increase of resistance to insulin has been observed in various infectious diseases, during allergic reactions, in hyperthyroidism, and hyperpituitarism (45b).

In severe insulin intolerance the blood sugar level may drop by more than 50 mg., and may even reach hypoglycemic levels of less than 20 mg. per hundred cubic centimeters (Fig. 18). Since such hypoglycemic reactions may produce shock, they should be terminated as soon as they become manifest, by the injection of 0.5 mg. epinephrine subcutaneously and intravenous administration of glucose.

The steep blood sugar curves produced by intolerance or hypersensitiveness to insulin are characteristic of hyperinsulinism. True hyperinsulinism is rare in children, occurring only in cases of adenoma of the pancreas and in the physiologic hyperactivity of the Langerhans islets in the newborn. Insulin intolerance in children, therefore, may in general be accepted as a sign of relative hyperinsulinism. According to Hartmann and Jandon (44b), it points to the possible presence of any one of the following clinical disorders: endocrine imbalance, adrenal or pituitary insufficiency, gastrointestinal disturbances with diminished absorption, starvation, liver anomalies, and organic intracranial lesions.

Children with recurrent attacks of hypoglycemia are abnormally sensitive to insulin, even during periods between attacks, when the blood sugar is at a normal level.

Normal newborn infants, in whom hypoglycemia is physiologic, have a definitely low tolerance (44b); their reaction to a test dose of insulin is on the borderline between normal and abnormal, with the abnormal predominating (Fig. 18).

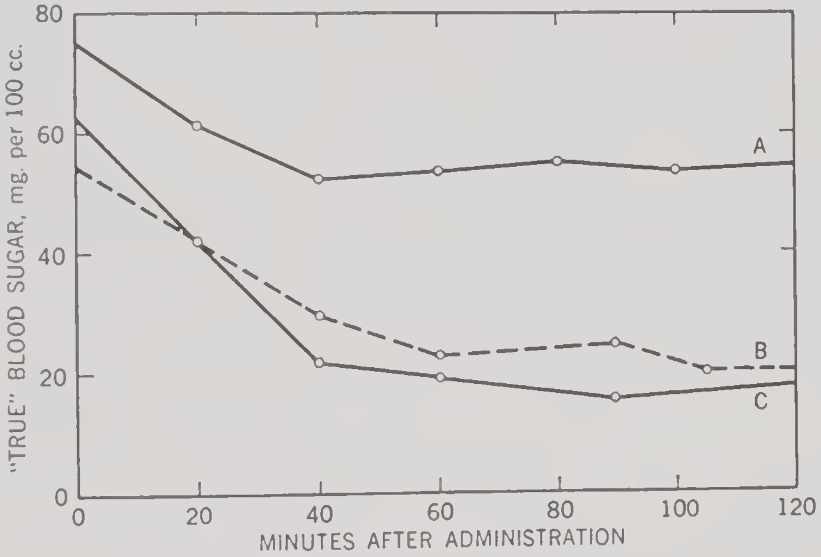


Fig. 18. Insulin tolerance test. Average blood sugar responses. A: Normal infants and children. B: Normal newborn. C: 3–5 year old children with recurrent severe hypoglycemic symptoms. After Hartmann and Jandon (44b) and Daniels (46a).

GLUCOSE–INSULIN TOLERANCE TEST

The simultaneous administration of glucose orally and insulin intravenously has been proposed by Himsworth (46b-c) as a test of insulin sensitivity in diabetic patients. The primary purpose of this test is to determine whether "a hyperglycemia unresponsiveness" (46d) is due to lack of insulin or resistance to it. The combined administration of glucose and insulin eliminates the influence of the fasting blood sugar level upon the outcome of either test.

Dosage of insulin and glucose is so balanced that the blood sugar curve remains almost unaffected (flat curve) in a normal person, in whom the normal responsiveness to insulin permits the test dose of insulin to neutralize the hyperglycemic effect of the sugar dose. But in a patient who is resistant to insulin, the postingestive hyperglycemia will not be counteracted by the dose of insulin, and a steadily rising hyperglycemic curve results.

The recognition of sensitivity or resistance to insulin is of great value in the study of diabetes. The test has also revealed interesting facts about other endocrine disorders of carbohydrate metabolism (46d).

While the test was devised for and has been used in adults, it would seem to be as useful in diabetic children. However, the special requirements obtaining in children must be kept in mind, as pointed out on pages 103 and 120.

PROCEDURE

Himsworth and Kerr (46c), the authors of the test procedure, make it clear that in testing diabetic subjects it is important that the diabetes be in a state of control prior to the test, i.e., absence of glycosuria except in the "after-breakfast specimen."

The test is begun in the morning, the patient having received neither food nor insulin during the preceding 12 hours. The test dose of glucose (page 104) is given by mouth, and the insulin is injected intravenously at the same time. The dosage is $^1/_{10}$ unit of insulin per kilogram of body weight. Blood is withdrawn immediately before, and 20, 30, 45, 60, and 90 minutes after administration of the test substances. Caution must be exercised in administering any given dosage of insulin. In patients with potential hypersensitivity to insulin, the test should be started with only $^1/_2$ to $^1/_3$ of the calculated test dose, and the appearance of hypoglycemic signs must be carefully watched for.

INTERPRETATION

Figures 18a and 18b illustrate the results obtained in different conditions. The curves shown are all adjusted to the same resting level by algebraic addition of the difference between the blood sugar value of 100 mg. per hundred cubic centimeters and the blood sugar level actually found in the first specimen. The results of the

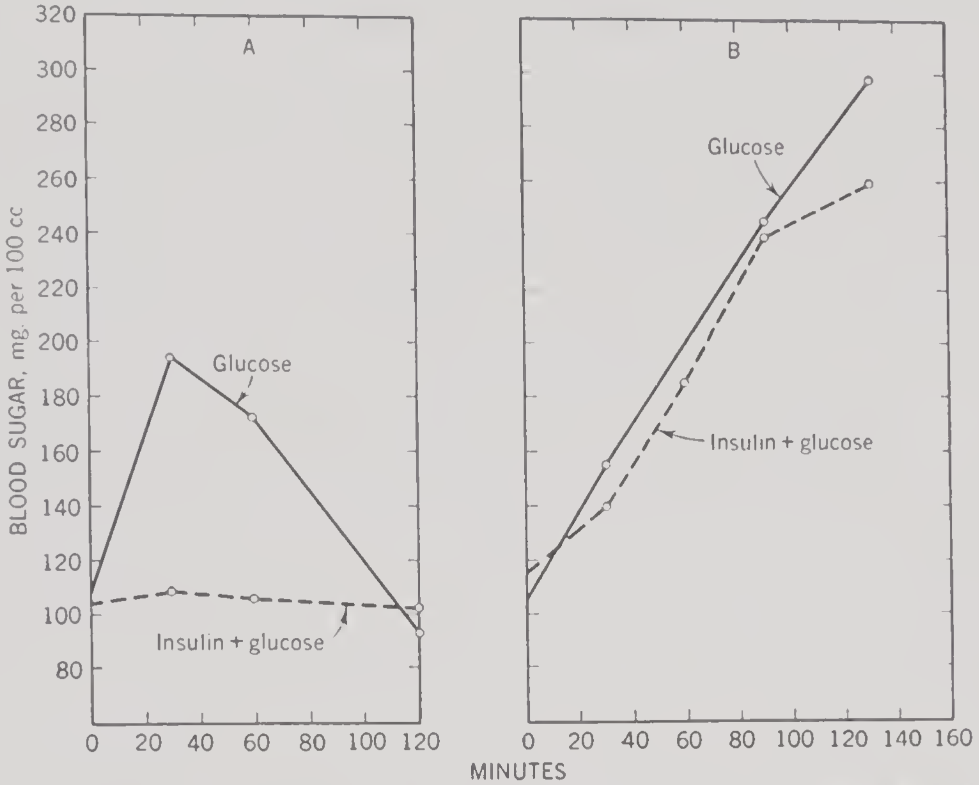


Fig. 18a. Glucose tolerance and glucose–insulin tolerance curves. A: Normal adult. B: Patient with Cushing's syndrome. From Fraser *et al.* (46d).

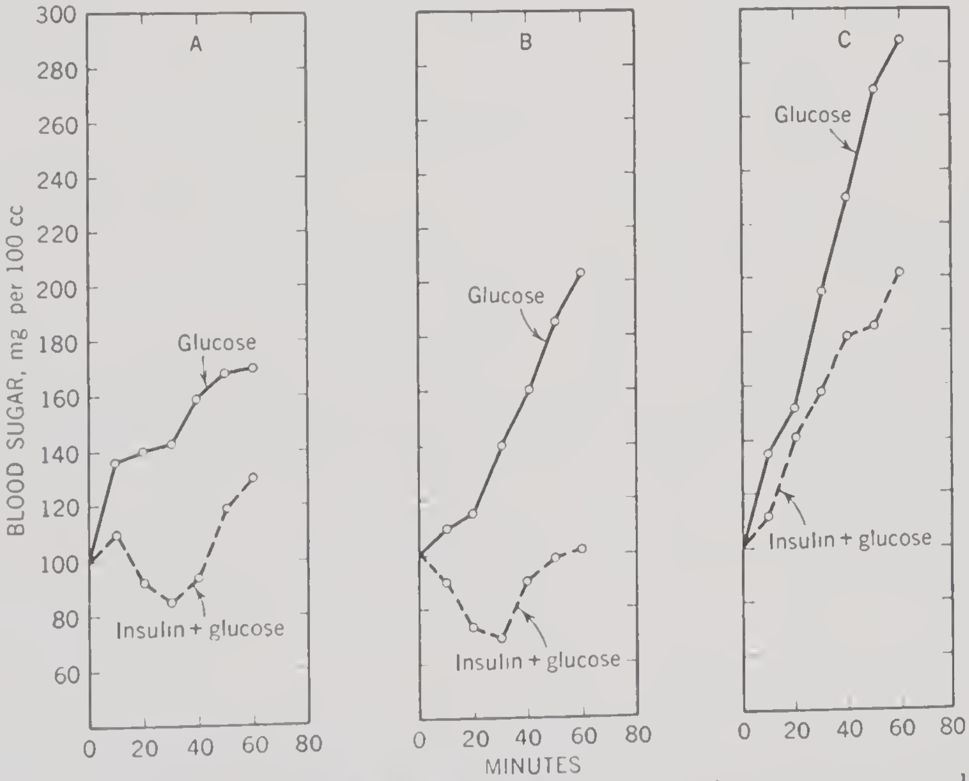


Fig. 18b. Glucose tolerance and glucose–insulin tolerance curves, charted as starting from a resting blood sugar level of 100 mg. per 100 cc. A: Normal adult; actual resting blood sugar levels, 91 and 102 mg. per 100 cc. B: Insulin-sensitive diabetic; actual resting blood sugar levels, 240 and 247 mg. per 100 cc. C: Insulin–insensitive diabetic; actual resting blood sugar levels, 173 and 170 mg. per 100 cc. From Himsworth and Kerr (46e).

glucose tolerance test alone and of the combined glucose–insulin tolerance test are given in each instance, but only the latter will be discussed here.

In a normal individual the blood sugar curve remains approximately flat after the combined test (Fig. 18a).

Diabetics who are sensitive to insulin show either the same type of flat curve as normal persons, or a hypoglycemic response of varying degree (Fig. 18b). This identifies the diabetes as a type which (*1*) is due to lack of insulin, (*2*) is responsive to insulin, and (*3*) is apt to develop ketosis rapidly if treatment is inadequate.

Diabetics who are insensitive to insulin respond to the combined test with a hyperglycemic reaction which is almost identical to that obtained with the glucose tolerance test alone (Fig. 18b). Such reactions indicate that the disease is probably due to overactivity of the anterior pituitary gland, with resultant insulin resistance.

The responses obtained in Cushing's syndrome are quite similar to the glucose–insulin test curves shown by diabetic patients insensitive to insulin (46d). This indicates that the high insulin tolerance in Cushing's disease is not due to lack of insulin but to resistance to insulin.

EPINEPHRINE (ADRENALIN) TEST

Parenteral administration of epinephrine results in hyperglycemia by causing a more rapid breakdown of liver glycogen and reducing the tissue utilization of sugar (47). The glycogenolytic action of epinephrine has been utilized for testing the availability of liver glycogen for metabolic oxidation. A hyperglycemic response to epinephrine proves that the liver is able to mobilize sugar from its glycogen stores, while failure to produce such a response reveals a lack of available glycogen.

Epinephrine and insulin are antagonists in their effect upon the blood sugar level.

The test as performed in adults needs no special adaptation for children.

PROCEDURE

The child should be in a fasting state when the test is made. The standard dose is 0.3–0.5 mg. of epinephrine or 0.3–0.5 cc. (5–10 minims) of a 1:1000 epinephrine solution, injected subcutaneously or intramuscularly. Untoward reactions, such as a sudden fall in blood pressure, or shock, must be watched for.

Blood specimens are taken before and 15, 30, 60, and 120 minutes after administration of the test dose. Urine is collected during the hour preceding epinephrine administration and for 4 consecutive periods of 2 hours each after the injection. The blood is analyzed for blood sugar by one of the methods already described, and each of the urine specimens is analyzed for acetone (page 152).

INTERPRETATION

The normal response to administration of a test dose of epinephrine is a rise in the blood sugar of 30 to 45 mg. per hundred cubic centimeters during the first hour after administration. An increase of only 20 to 30 mg. may be considered subnormal, and an increase of less than 20 mg. is definitely abnormal and a sign of high epinephrine tolerance. Hypersensitiveness to epinephrine is indicated by a rise of more than 45 mg. per hundred cubic centimeters.

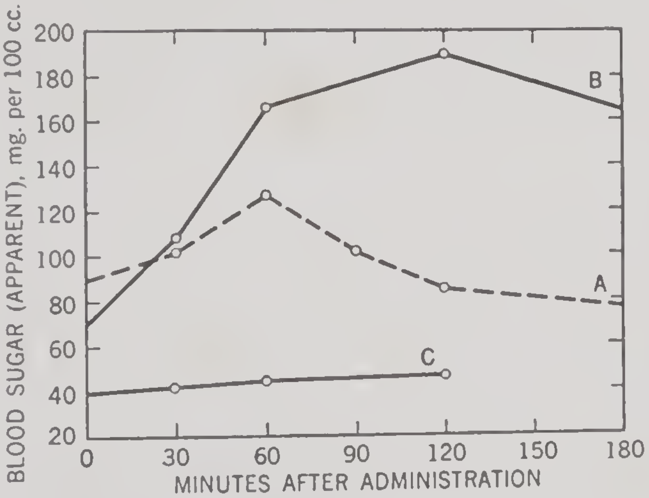


Fig. 19. Effect of adrenalin upon blood sugar. A: Response of a normal 5 year old child. B: Abnormally strong response of a 3 month old infant with glycogen storage disease and cretinism; after Hertz (52). C: Lack of response of a 5 year old child with glycogen storage disease; after van Creveld (15).

An abnormally weak response may indicate: (*1*) Depletion of the glycogen stores in the liver; clinical examples responsible for such a condition are organic liver diseases, e.g., cirrhosis or fatty infiltration, and chronic hypoglycemia of hepatic origin (44b). (*2*) Inability of the liver to mobilize the glycogen stores, although they are ample. This defective glycogenolysis is clinically exemplified in such conditions as glycogen storage disease (48a), some rare cases of pituitary hypoglycemia, and severe hyperinsulinism (48b). The weakened response to epinephrine may be distorted or reversed if other disturbances, particularly of endocrine nature, are also present. Figure 19 illustrates the typically weak reaction to epinephrine in glycogen storage disease, and the unusual response of a child with this disease and hypothyroidism.

The significance of abnormally high and persistent reactions to epinephrine is still a matter of conjecture. For example, children with cyclic vomiting and recurrent attacks of hypoglycemia are hypersensitive to epinephrine (44,49,50). Wilder (51) may be right when he cautions against attributing too much diagnostic significance to the blood sugar reaction to epinephrine.

Acetonuria has been reported following injection of epinephrine in children with glycogen storage disease (48). The occurrence of such a ketogenic effect supports the diagnosis of glycogen disease, since other chronic hepatogenic disorders of carbohydrate metabolism do not react with acetonuria (Table 27), despite an equally weak blood sugar response.

DETERMINATION OF GLYCOGEN IN BLOOD

PROCEDURE

The method described is that of van Creveld (53). 1 cc. of blood is transferred into a wide, glass-stoppered pyrex centrifuge tube and hemolyzed by adding 1 cc. of distilled water. 2 cc. of 60 per cent potassium hydroxide are added, the tube is stoppered, and placed in a boiling water bath for 15 to 20 minutes, with occasional shaking during the incubation period. The contents are allowed to cool, 8 cc. of distilled water and 16 cc. of absolute or 96 per cent alcohol are added, the contents are carefully mixed, and the precipitated glycogen is allowed to settle overnight. The tube is then centrifuged, and the precipitate is washed at least twice with 66 per cent alcohol. The alcohol is evaporated, 4 cc. of 2.2 per cent hydrochloric acid are added, and the tube is incubated at 37 C. for 2 hours. The material is then neutralized with 2 *N* sodium hydroxide, using 1 drop of phenol red as indicator. Determination for glucose is carried out in an aliquot of this solution by one of the methods already described.

INTERPRETATION

According to Bridge and Holt (54), the normal glycogen content of whole blood in children under 12 years of age, expressed as glucose, is less than 20 mg. per hundred cubic centimeters, in the majority ranging between 10 and 15 mg. An increased blood content of glycogen is one of the diagnostic criteria of glycogen storage disease (53,54). Mean values ranging from 21.2 to 85 mg. per hundred cubic centimeters have been observed. The authors state (54): "It seems probable that the increased level of blood glycogen represents in reality an increase in one of the tissues of the body, namely the leukocytes."

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CHAPTER V

Fat Metabolism Tests

Fatty substances or lipids are found in all body cells. Their amounts vary widely; in bone marrow the concentrations are particularly high, constituting up to 65 per cent of the weight of the fresh organ. The following scheme, adapted from Lehnartz (1a), shows the chemical relationship between the various substances known to constitute the lipids of the human body.

Cholesterol ester	cholesterol	
	fatty acid	neutral fat
Lecithin and cephalins*	glycerol + 2 molecules fatty acid	
	phosphoric acid + choline	sphingomyelin
Kerasin and other cerebrosides	sphingosine + fatty acid	
	galactose	

Functionally considered, there are two groups of fats (2)—depot fats and organ fats. Depot or reserve fat consists largely of fatty acids and neutral fat, located chiefly in subcutaneous and intramuscular connective tissue and in the omentum, and serves as the main energy store of the body. The amount and composition of depot fat is variable, depending on metabolic and dietary factors. This fat can no longer be considered as inert. According to Longenecker (2), "There is continual replacement of the constituent fatty acids by new ones which may be either exactly the same structually as the displaced components or entirely different. . . . A continual source of fatty acids is available for this dynamic metabolism of the fat tissue either from food fat or by synthesis de novo."

The second group, i.e., organ fats, are largely made up of phospholipids and cholesterol. These two fat components are widely distributed in the body and are an essential constituent of all cells. Sphingomyelin is found in large amounts in brain and nerve tissue. Though organ fats participate in metabolic processes (3), their amounts and composition tend to remain constant. Of their function in the animal body, little is known; Sinclair (3) believes that phospholipids may play an essential part in the structural make-up of cells and cell membranes, in the transportation and metabolism of the fatty acids, and in the mechanism of intracellular respiration.

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* Cephalins have colamine instead of choline united to the glycerol phosphate radical; serine or inositol are also present in cephalins (1b).

The physiologic significance of cholesterol is still a matter of conjecture. There is evidence that it takes an active part in the digestion and absorption of fat (bile cholesterol) and that it is of importance in several physiologic relationships, such as hemolysis and settling of red corpuscles (4). There is also a close relationship to vitamin D.

Knowledge is likewise lacking as to the various phases of lipid metabolism, primarily of the factors that control the metabolic fate of lipids after they have been absorbed by the intestines and have entered the lymph and blood stream. The liver plays a prominent role in the transformation of fats into energy, in the synthesis of phospholipids, and in the formation of cholesterol esters. Under normal conditions, lipids are found in the feces but not in the urine, with the exception of cholesterol, which may be present in normal urine (5a).

Several facts should be kept in mind when quantitative tests for blood lipids are used, as Bloor (4) points out. (*1*) There is ordinarily a well-regulated balance in the blood between inflow and outflow of lipids, which keeps the fat at a low level. (*2*) Increase of free fat in the blood is always followed by an increase in phospholipid and cholesterol. This connection may be of metabolic nature, but possibly may only be related to fat transport. (*3*) Only plasma lipids change freely with inflow and outflow of fatty material; the composition of the corpuscles is affected slowly, if at all. Determinations of blood lipids, therefore, should be made solely on plasma.

From the foregoing it may be seen that many processes of fat metabolism and many conditions which affect the level of blood lipids still remain obscure. The interpretation of the various quantitative tests of fat tolerance therefore is still a matter of debate. From the mass of available literature the author has attempted to assemble here the data on test procedures which might be most helpful in the clinical study of fat metabolism in children. These procedures include: (*1*) Microscopic and chemical examination of feces (page 20) to ascertain intestinal absorption and fermentation of fat. (*2*) Examination of fasting blood for fat, to ascertain intestinal absorption, transportation, and assimilation of fat. (*3*) Estimation of total cholesterol and of cholesterol partition, to ascertain intestinal fat absorption, assimilation, and ester synthesis. (*4*) Examination of changes in fat content of blood, in response to an oral test dose of fat. The results of this test provide information on intestinal absorption, assimilation, and storage of fat. (*5*) Examination for the ketonemic response to an oral test dose of fat. The curve obtained in such a test gives a clue to fat utilization and perhaps to the tendency to ketosis. (*6*) Carbohydrate deprivation test, which demonstrates the capacity to prevent ketosis.

BLOOD LIPIDS ASSAY

The degree of lipemia can be estimated roughly by microscopic counts of "chylomicrons" in dark field preparations (6a-c) and by the volumetric lipocrit method (7). Chemical micromethods are available for exact quantitative determinations of blood lipids.

The fatty materials of blood plasma consist of (*a*) fatty acids, (*b*) neutral fat, (*c*) phospholipids, (*d*) free cholesterol, (*e*) combined cholesterol (esters). Each of these, a combination of them, or their sum total, may be determined or computed. Fractionation of blood lipids is based upon their different solubilities in alcohol, ether, and acetone. While Boyd (8) has developed an analytic procedure for complete fractionation of the plasma lipids, it is a difficult procedure for serial determinations in children, since it requires 10 cc. of plasma. Bloor's method (9) for determining fatty acids plus cholesterol, or the total fatty acids, presents fewer technical difficulties, requiring only 3 cc. of plasma. Neutral fat plus free cholesterol can be determined in the primary ether extract by the micromethods of Bang (10) and of Bing and Heckscher (11,12); these methods require only 0.1 cc. of capillary blood. Kirk, Page, and Van Slyke's gasometric microanalysis (13) requires 3 cc. of plasma for determination of all lipid fractions. Cholesterol and its partition can be determined by Bloor's method (9) with 1 cc. of plasma, or by Schoenheimer and Sperry's method (23,24) with 0.2 cc. of serum.

The normal fasting level of blood fats varies widely in individuals of identical age, as well as in any one individual; this holds true for the total amount of plasma lipids and for the different fractions. Normal mean values that have been reported for infants and children are in Table 28 (p. 142), together with the accepted normal values for adults. The lower values of all lipid fractions during the first weeks of life are clearly apparent. While we know that all values become stabilized at adult levels prior to puberty, data on plasma lipid values during childhood are still too scanty

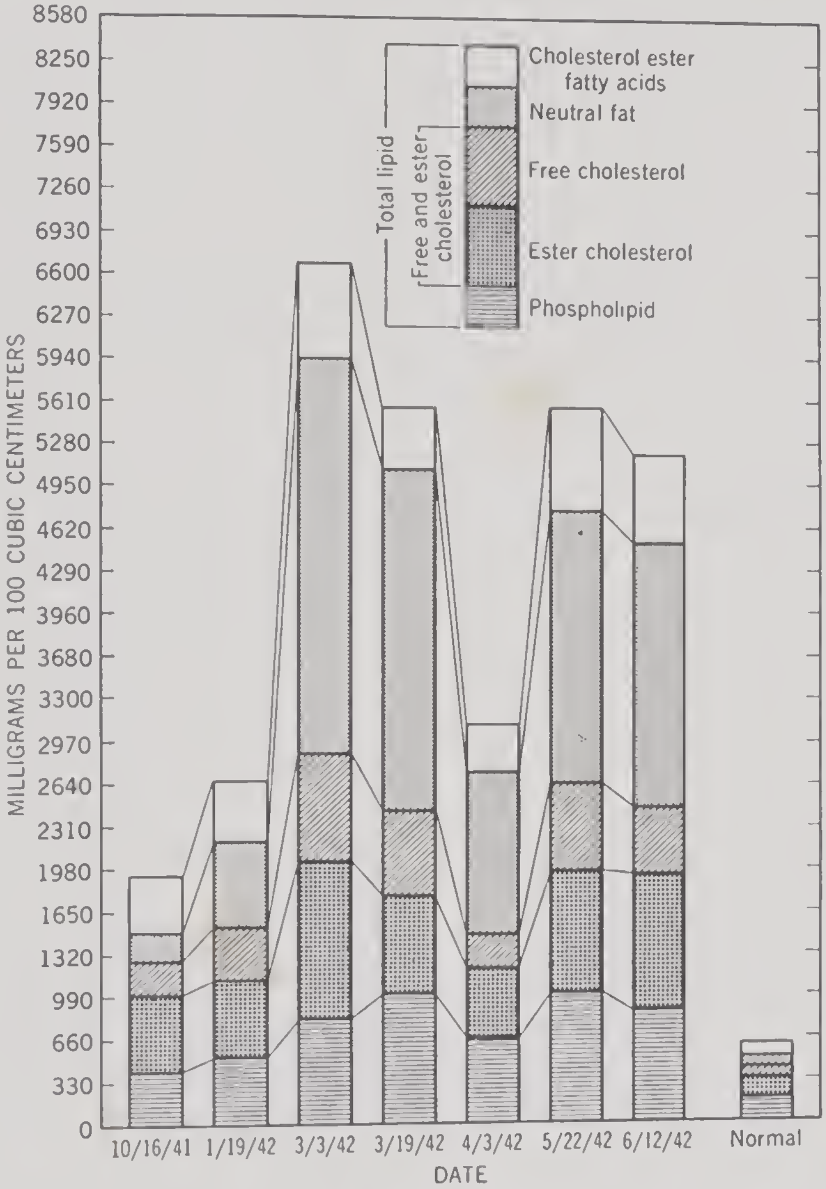

Fig. 20. Changes in total and fractional lipid levels in plasma during progress of nephrotic syndrome. From Thomas (16).

for establishing an age curve from birth to puberty. It can safely be said, however, that the most significant changes toward the normal adult level take place within the first few months of life.

Children with diabetes, leukemia, and severe hemolytic anemias show abnormally high plasma lipid levels, far exceeding the normal adult values (16). For the pediatrician, the hyperlipemia of the nephrotic syndrome (16), of hypothyroidism (17), of some of the lipoidoses (18), and of glycogen storage disease (19,48) is of particular interest. Carbohydrate starvation, as induced by a ketogenic diet, also causes a severe hyperlipemia (20).

Figure 20 illustrates the extent to which the various lipid fractions may participate in the total increase. Thus, in the early stages of the nephrotic syndrome free and combined cholesterol are chiefly responsible for the high total lipid values, while during the late phases it is a rise in neutral fat which accounts for most of the increase.

Abnormally low plasma lipid levels have been observed in hyperthyroidism (21). Values also tend to be depressed in the celiac syndrome; Parsons (22) found that the total lipids averaged 474 mg. per hundred cubic centimeters, as compared with the average normal of 617 mg.

As to the functional significance of abnormal levels of blood fat, all one can say is that they indicate a disturbance in some or all of the various phases of fat metabolism. Impairment of a particular stage of the metabolic process may sometimes be inferred from other evidence. For the diagnostic significance of the ratio of free to combined cholesterol, see page 41.

ASSAY OF TOTAL, FREE, AND COMBINED CHOLESTEROL IN BLOOD SERUM

The method described is that of Schoenheimer and Sperry (23,24).

Apparatus and Reagents.

(*1*) Electrophotocolorimeter.

(*2*) A mixture of 1 part redistilled acetone and 1 part redistilled absolute alcohol.

(*3*) A mixture of 1 part redistilled acetone and 2 parts peroxide-free ether.

(*4*) Digitonin solution. Dissolve 400 mg. digitonin in 100 cc. distilled water; if not clear, filter or centrifuge before use.

(*5*) Potassium hydroxide solution. Dissolve 10 Gm. pure potassium hydroxide in 20 cc. water. Store in a dropping bottle; if a sediment develops, filter through sintered glass before use.

(*6*) Glacial acetic acid.

(*7*) Acetic acid solution. Dilute 10 cc. glacial acetic acid to 100 cc. with water.

(*8*) Acetic anhydride–sulfuric acid reagent. This must be prepared immediately before use. Transfer, under constant agitation, 20 cc. chloride-free acetic anhydride and 1 cc. concentrated sulfuric acid into a glass-stoppered flask placed in an ice bath. Stopper the flask, shake vigorously, and return to the ice bath.

(*9*) Cholesterol stock solution. Dissolve 100 mg. cholesterol in glacial acetic acid and make up to 100 cc. with the solvent.

(*10*) Cholesterol standard solution. A mixture of 1 part cholesterol stock solution and 9 parts glacial acetic acid. 1 cc. of the solution contains 0.1 mg. cholesterol.

PROCEDURE

Extraction. For a single determination, 2 cc. of the acetone–alcohol mixture are placed in a 5 cc. volumetric flask, 0.2 cc. of serum is added from a capillary pipet, allowing the serum to run down the wall of the flask, and the mixture is swirled vigorously. A finely divided precipitate should result. The flask is placed over a steam bath until the solvent boils, and during this interval the flask should be rotated to prevent dumping. After the flask is cooled to room temperature, acetone–alcohol mixture is added to make up to volume, the contents are thoroughly mixed, and then filtered through a small, dry filter into a test tube. The filtrate is immediately anaylzed for free cholesterol and/or total cholesterol.

Precipitation of Free Cholesterol. Two cc. of the filtrate are pipetted into a 12 cc. centrifuge tube, 1 cc. digitonin solution is added, the mixture is stirred with a glass rod, which is then left in the tube, and the tube is placed in a preserving jar. The jar is tightly covered and left overnight at room temperature. The tube is then removed and placed in a test-tube rack. The solution is stirred gently, to free particles that may have adhered to the wall of the tube, and the stirring rod is carefully removed without touching the upper part of the tube and laid on a rack of heavy wire without rubbing off adherent precipitate. The tube is centrifuged until the precipitate is so tightly packed that the supernatant liquid can be decanted off without loss. The loss of the few small particles that usually float on the surface and cannot be centrifuged down does not affect the results. If any of the precipitate is seen to be suspended in the fluid during decanting, centrifuging must be repeated. After decanting, the tube is drained for a few minutes; the last drop is removed by touching the lip of the tube to a clean towel. The stirring rod is replaced in the tube; the wall of the tube and the rod are washed down with 1.5–2 cc. of the acetone–ether mixture from a dropping pipet. The precipitate is stirred up, the rod is removed to the wire rack, the tube is centrifuged for 5 minutes, and the centrifugate is decanted as before. The washing of the precipitate is repeated two more times, using ether instead of the acetone–ether mixture. The solution is then decanted, the stirring rod is replaced in the tube, and the last traces of ether are drawn off with the aid of a pipet attached to a suction pump. The sample is now ready for color development.

Precipitation of Total Cholesterol. One cc. of the acetone–alcohol extract of serum is pipetted into a 12 cc. graduated centrifuge tube; 1 drop of potassium hydroxide solution is added, stirred into the extract with a rod, and the rod left in the tube. The tube is placed in a preserving jar containing a layer of about 3 cm. of sand which has been heated to about 45 C. by placing the jar in a water bath, the jar is tightly covered, and is then incubated at 37 to 40 C. for 30 minutes. The tube is removed to a rack and allowed to cool. The stirring rod is then raised, acetone–alcohol solution is added to the 2 cc. mark, and the contents are titrated with 10 per cent acetic acid solution, with 1 drop of a 1 per cent alcoholic phenolphthalein solution as indicator. From 4 to 6 drops of acetic acid solution are required, with 1 drop added in excess. Then 1 cc. of digitonin solution is added, and the solution is thoroughly stirred. The tube is replaced in the preserving jar, which is tightly covered and left at room temperature for at least 3 hours, but preferably overnight. The precipitate is then centrifuged and washed, in the same manner as in the determination for free cholesterol, but only one ether washing is necessary. The sample is now ready for color development.

Color Development and Reading. A shallow pan containing about 3 cm. of sand is heated to 110–115 C. in an oven. The tubes containing precipitate and rod are placed in the sand in order of reading, and the pan is returned to the oven for 30 minutes. A water bath is adjusted to 25 C. during this interval.

Sperry (24) recommends that incubation be carried out in a dark cabinet. A small wooden packing box, equipped with a door, serves the purpose well. A copper pan, about 4 inches high, with a wood cover with holes for centrifuge tubes, should fit into the box, and provision should be made for a thermometer and a funnel for adding hot or cold water, as may be necessary.

The pan is removed from the oven; 1 cc. of glacial acetic acid is pipetted into the first tube, the acid being allowed to wash down the rod and the wall of the tube, the contents are stirred vigorously, and the tube is left in the hot sand while acid is being added to the next 2 or 3 tubes. This takes 2 to 3 minutes in all. The contents of the first tube are stirred again, the tube is removed from the sand, allowed to cool, and placed in the water bath. The acetic anhydride–sulfuric acid reagent (page 138) is now prepared. When the reagent has been in the ice bath for about 9 minutes, the first tube is removed from the water bath and 2 cc. of the reagent are added. The mixture is stirred for a few minutes, the rod is removed, and the tube is returned to the water bath. The color reading may be made at any time between 27 and 37 minutes after the reagent is added. In the same way reagent is added to each of the other tubes, in order.

A blank is run containing 1 cc. of glacial acetic acid and 2 cc. of the acetic anhydride–sulfuric acid reagent.

The standard contains 1 cc. of the standard cholesterol solution and 2 cc. of the acetic anhydride–sulfuric acid reagent.

For readings with the Evelyn electrophotocolorimeter, the microunit should be installed. Open cells may be used. Filter No. 660 is inserted. The cuvet is filled with the blank solution and resistance is adjusted so that the galvanometer reads 100. The center setting is established, after which the unknown samples are read and the galvanometer readings are recorded.

Calculation. Total cholesterol minus free cholesterol equals cholesterol ester.

$$\text{mg. free cholesterol per 100 cc. serum} = 125(d_u/d_s)$$

$$\text{mg. total cholesterol per 100 cc. serum} = 250(d_u/d_s)$$

where d_u and d_s are the densities of the unknown and of the standard, respectively

The density is either read directly from the scale of the apparatus (in some of the models), or calculated from the percentage transmittance (as with the Evelyn electrophotocolorimeter) by the equation:

$$d = 2 - \log G$$

where G is the galvanometer reading.

If this method is used routinely, prepared calibration curves for cholesterol are a convenience, since they permit direct reading of the cholesterol concentration in milligrams per hundred cubic centimeters of serum when the galvanometer reading is known.

Calibration. The following description of the preparation of a calibration curve is taken from the directions for the use of the Evelyn colorimeter (25). Six sample tubes, each containing 1 cc. of a different cholesterol dilution and 2 cc. of acetic anhydride–sulfuric acid reagent, are set up. The seventh tube contains 1 cc. glacial acetic acid and 2 cc. of the reagent.

The dilutions of cholesterol are prepared from the cholesterol stock solution so as to contain 0.1, 0.2, 0.4, 0.6, and 0.8 mg. cholesterol per cubic centimeter. These amounts correspond, under the given analytic conditions, to serum cholesterol concentrations of 50 to 500 mg. per hundred cubic centimeters. The 6 samples and the blank are treated exactly as described above, and are read in the photocolorimeter. The galvanometer readings are plotted on semilogarithmic paper against the concentrations of cholesterol contained in the corresponding dilutions. The readings are charted on that side of the paper which is calibrated logarithmically.

INTERPRETATION

Normally, serum concentration of total cholesterol in children varies with age. In the newborn the level is only half or less than half, as high as in adults (Tables 28, 29). Increasing progressively through infancy and childhood (Table 29), the average cholesterol concentration in serum reaches low adult values between the ages of 5 and 7 years (15,16,26).

The studies of Hodges, Sperry, and Anderson (27) suggest a different course of the age curve of serum cholesterol. These authors found that the level rose to normal adult values by the age of 2 to 6 months, and that thereafter it remained practically unchanged throughout infancy, childhood, and adolescence (Table 29A).

The wide limits of the normal range are noteworthy. Sperry (28) states: "Unless the total cholesterol content of the serum of a patient is extremely low or high, one cannot be certain that the amount found is abnormal for that person."

TABLE 28
Composition of Plasma Lipids in Normal Subjects
(Mean Values in Milligrams per Hundred Cubic Centimeters)

Age	Total lipids	Neutral fat	Phospholipid	Cholesterol Total	Cholesterol Combined	Cholesterol Free
Newborn (14)*						
1–13 hours	221	80	27	114	82	32
6–10 days	468	173	103	193	142	51
Children (15)*						
5–9 years	454	100	136	143	—	—
Adults (8)†	589	153	195	162	115	47

Numbers in parentheses are reference numbers.

* Determined by procedure of Kirk, Page, and Van Slyke (13) in heparinized blood.

† Determined by Boyd procedure (8) in citrated blood.

TABLE 29
Normal Total Cholesterol in Serum in Relation to Age

Age	Total cholesterol, mg. per 100 cc. Range	Total cholesterol, mg. per 100 cc. Average
Newborn (cord blood)	54–120	79.3
1–30 days	90–160	122.4
2 months	87–169	126.5
3 months	106–184	138.4
4 months	142–178	154.4
5–12 months	125–198	159.1
2–6 years	144–188	172.7
6–13 years	150–250	190.2
Adults*	148–392	209.8

According to György (26).

* According to Sperry (28).

The total cholesterol values are abnormally high in conditions associated with an abnormal increase of blood lipids (page 134). Among such conditions are diabetes, nephrosis, aplastic anemia, hypothyroidism, biliary obstruction, and glycogen storage disease, and carbohydrate starvation.

Total cholesterol values have been reported as being abnormally low in severe anemia (29), hyperthyroidism (30), severe infections (31), and in a large proportion of the patients with hepatitis (32).

In individuals of similar age, the actual values of the component fractions, i.e., of free and ester cholesterol, show the same wide variations as the total cholesterol. The ratio of free to ester cholestrol, however, is relatively constant. Changes in the normal ratio are almost invariably the result of a greater decrease in the ester fraction than in the free fraction. Under physiologic conditions, a relatively low value for the ester fraction is found only during the neonatal period; it is one of the many signs of the functional immaturity of the newborn. With this one exception, an abnormally low ester fraction is evidence of functional liver damage. The various ratios which define the relation between free, ester, and total cholesterol, their normal variation with age, and their characteristic changes in hepatic insufficiency have already been discussed (pages 41–43).

TABLE 29A
Total Cholesterol Content of Serum of Normal Children

Age	Total cholesterol Mean, mg./100 cc.	Total cholesterol Standard deviation	Age	Total cholesterol Mean, mg./100 cc.	Total cholesterol Standard deviation
2–6 mos.	200.2	±49	7th	204.3	±32
7–12	206.6	±35.6	8th	204.3	±34
2nd yr.	203.2	±38	9th	220.4	±42
3rd	202.4	±30.5	10th	205.8	±34
4th	194.0	±29	11th	206.2	±42
5th	212.6	±38	12th	210.4	±42
6th	208.6	±39	13th	204.3	±32

From Hodges, Sperry, and Anderson (27).

BLOOD FAT LOADING CURVE

The course of one of the chief plasma lipid fractions is observed for 4 or more hours after oral administration of a standard dose of fat. Deviations from the average normal course point to a disturbance in fat metabolism. Unlike the blood sugar curve, which is principally an "assimilation curve," the blood fat loading curve depends on the rate of absorption, assimilation, and possibly mobilization. Whether abnormal curves may be interpreted as being due chiefly to variations in intestinal absorption is still open to question, although the test is often called and considered as a "fat absorption test."

PEDIATRIC CONSIDERATIONS

There are only a few studies on the reactions of children to the loading test. No adverse effects of test meals have been observed; occasionally, there is a change in color and consistency of stools. A relatively weak lipemic response is the chief peculiarity found in children, so that the test dose given adults (1 Gm. of fat per kilogram of body weight) must be doubled (33) in order to produce the same degree of lipemia. Furthermore, in children the curve has a different shape, showing a high initial phase and reaching a climax sooner than in adults (usually after 2 to 3 hours). For this reason, the most significant part of the curve may be missed when only one blood specimen is taken, 4 hours after the test meal, as recommended for adults (34). If the test dose is increased to 3 or more grams of fat per kilogram, the high phase of the curve lasts longer, but there are greater variations in attainment of the maximum level and the return to the starting level.

PROCEDURE

Nissen's method (33), here described, is to be used only for children over 2 years of age. The test meal, which contains 2 Gm. of fat per kilogram of body weight, is given after a fast and rest in bed of at least 12 hours. The meal consists of 1 zwieback or slice of white bread, a cup of tea, and cream to furnish the required amount of fat; about 6 cc. of cream per kilogram of body weight are required if the cream contains 32–33 per cent fat. A few grams of the necessary total fat may be given in the form of butter on the bread. Ingestion of the test meal may take as long as 20 minutes. The child should be kept in bed until the test is completed. One or two glasses of water may be given during the test.

Blood is drawn immediately before the test meal and every hour for at least 4 hours thereafter, the quantity of blood depending on the lipid fraction to be estimated and the method chosen.

Plasma lipids are estimated in each of the blood specimens, and the curve of total lipids or of one of the main lipid constituents is thus obtained.

INTERPRETATION

Figure 21 shows several alimentary blood fat curves, representing the changes in the concentration of the primary ether extract fraction. From curve *A* it can be seen that in normal children the rise in the fat level is definitely noticeable an hour after ingestion of the test meal. Usually the highest point is reached between the second and third hours; thereafter it declines, returning to the fasting level about 4 to 5 hours after the test meal. Generally, but not invariably, the maximal increase in lipid concentration does not exceed the fasting value by more than 50 per cent; but a rise of 100 per cent has also been observed in healthy children. Obviously, there is a marked variation, even with the standard loading dose. This is also true of the curves of total fatty acids and total lipids. The variability of all fat tolerance curves is so great that only extremely abnormal curves should be considered as having pathognomic significance.

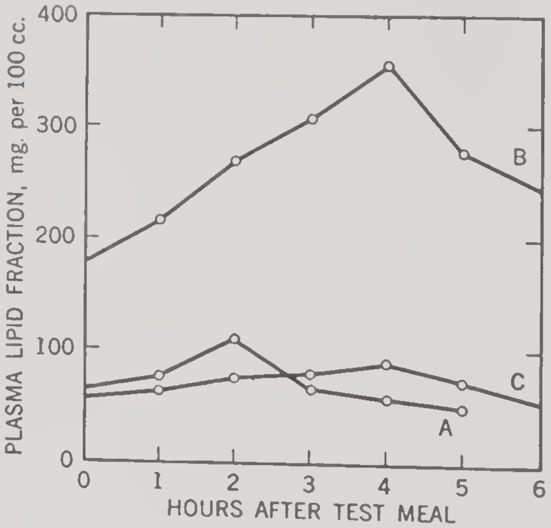

Fig. 21. Fat loading curves in plasma, showing concentration of primary ether extract fraction of lipids. A: Normal; mean values obtained in 5 healthy children, 2 to 12 years old. B: High rise in a 2 year old child with nephrosis. C: Normal; mean values obtained in 13 healthy adults. From Nissen (33).

The curve in adults is distinctly different (Fig. 21); the peak is reached between the third and fourth hours, and the return to the fasting level is completed between the fifth and seventh hours.

In children, a second standard loading dose given a few hours after the first one has no influence whatever on the lipemic curve, or merely protracts its course. This is in contrast to the reaction of adults, who show a renewed rise when the test meal is repeated.

An increase of more than 100 per cent above the initial value is considered abnormal. Such strong reactions denote fat intolerance. The high rise may signify that fat is supplied to the blood at a faster rate than it is removed by assimilation. In children it is mainly the nephrotic syndrome which produces these abnormally high curves (Fig. 21). Little is known regarding the occurrence of high curves in other diseases of children, including hepatic disorders. Although the liver is assumed to have a key position in maintaining a normal blood lipid level, the relationship between hyperlipemic response and liver function is still a matter of conjecture.

Flat curves, i.e., absence of any considerable hyperlipemic reaction, are considered evidence of fat intolerance due to impaired intestinal absorption, provided the test dose was adequate. The supply of fat to the blood is decreased or blocked, but the power to assimilate is intact. Such curves (Fig. 22) are obtained in the celiac syndrome (22, 35), though not regularly. Published information on this subject is scanty. A better demonstration of defective intestinal absorption in the celiac syndrome is obtained by the vitamin A absorption test (page 257).

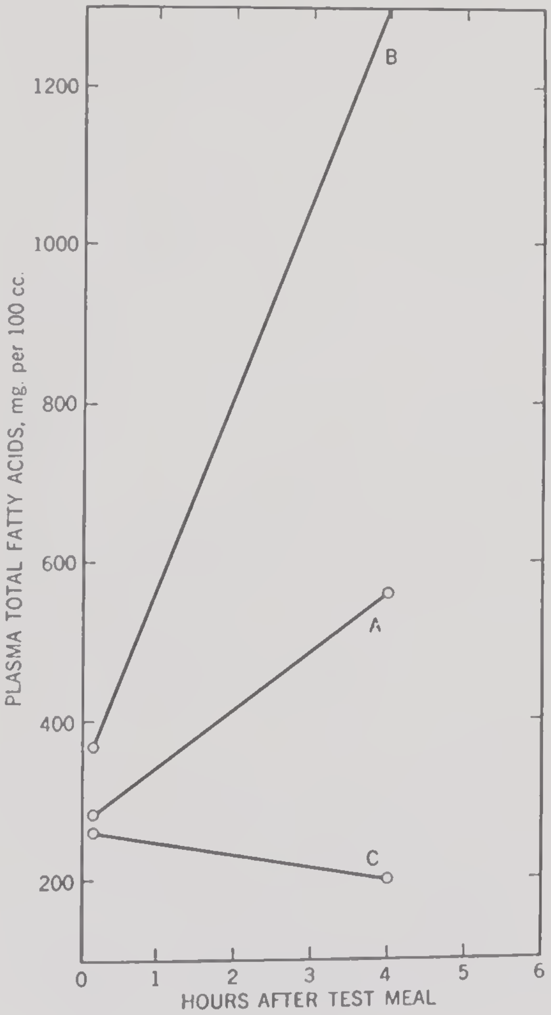

Fig. 22. Fat loading curves in plasma, obtained in children with celiac syndrome, showing concentration of total fatty acids after a test meal of 4 ounces of 35 per cent cream. A: Normal response. B: Abnormally high curve, indicating low tolerance. C: Flat curve, indicating impaired intestinal absorption. Based on data of Luzatti and Hansen (35).

KETONEMIC CURVE AFTER A TEST DOSE OF FAT

As noted above, neither the normal nor the abnormal blood fat loading curves are very consistent. In a search for more reliable methods, Kauvar (36) devised a fat tolerance test in which the level of blood ketones serves as a criterion of the power to assimilate fat. The ketonemic curve is determined after ingestion of a test meal consisting of 35 Gm. of butter on 15 Gm. of gluten bread, taken after an overnight fast. Total ketones are determined in blood samples taken before and 2, 3, and 5 hours after the test meal. A convenient method is the microdetermination of acetone and diacetic acid in 0.2 cc. of blood or serum (37).

Kauvar reports that in normal adults, including a few older children, with a fasting ketone level not exceeding 1 mg. of total acetone per hundred cubic centimeters, no hyperketonemia occurs after the test meal and the curve remains flat. In patients with myxedema and cretinism, the ketonemic curve rises considerably between the first and third hours after the test meal. All the patients had high fasting levels of blood lipids (cholesterol). In various endocrine disorders, all of them showing an abnormally high ketone concentration in the fasting blood sample, the ketonemic curve declines to subnormal levels during the first 3 hours after the test meal and then rises again to the high initial values (Table 30).

TABLE 30

Ketonemic Response to Standard Fat Meal under Normal and Pathologic Conditions

Condition	Total blood ketones, as mg. acetone per 100 cc.			
	Fasting	After 2 hours	After 3 hours	After 5 hours
Normal................	1.0	1.0	1.0	1.0
Hypothyroidism........	1.0	11.9	2.0	1.0
Hyperthyroidism.......	5.2	1.0	1.0	1.0
Frölich's syndrome.....	9.4	1.0	1.0	4.3
Lorain-Levi syndrome...	6.6	1.0	1.0	3.9

After Kauvar (36).

Kauvar favors the hypothesis that the ketonemic reaction is primarily caused by a pituitary response to the fat meal, decreased, increased, or normal secretion of ketogenic hormone producing the respective patterns of the ketonemic curve.

No reports on the response of young children to the test have been found.

CARBOHYDRATE DEPRIVATION TEST

The liver is the principal, perhaps the only, site of formation of ketone bodies (acetoacetic acid, beta-hydroxybutyric acid, acetone), and fat appears to be their principal source (5b). Under normal conditions, the capacity of many peripheral tissues to dispose of ketones prevents their accumulation in blood and tissues. If, however, there is an increase in ketone production as compared to ketone utilization, an abnormal rise in blood ketones results. It is now believed (38,39) that an excess of ketones in blood and tissues is due to an abnormally increased rate of ketone formation in the liver.

In the intact organism carbohydrate forms the chief substrate for oxidation by the hepatic cells; it is the primary antiketogenic substance, by virtue of its ready availability from glycogen and its ability to suppress the oxidation of fat and the formation of ketones. Proteins, on the other hand, are not as antiketogenic. Ketosis appears whenever fat wins the competition for oxygen in the hepatic cells. A tendency to oxidize fat in preference to sugar may appear in various conditions: in diabetes, during acute infections and following surgical intervention. In normal subjects this tendency may appear during a fast or if carbohydrate intake is greatly restricted, and after administration of adrenalin or anterior pituitary extract. Adrenocortical extract will stimulate antiketogenesis. In the diabetic patient, insulin will stimulate it.

With respect to diet, ketogenesis depends on the ketogenic balance of the foodstuffs being oxidized in the body (40). The diet normal for the age provides a surplus of antiketogenic substances, thus inhibiting ketogenesis. Ketosis sets in as soon as the ratio of ketogenic to antiketogenic foodstuffs exceeds the individual's tolerance. The shift from oxidation of carbohydrate to oxidation of fat can be demonstrated by the postulated drop in respiratory quotient (Fig. 25, page 153).

Two ratios express the ketogenic balance of foodstuffs; one is the fatty acid–glucose ratio (F.A./G.), the other is the ketogenic–antiketogenic ratio (K./A.). The value of the first ratio is approximately 1.5 times that of the second. Both ratios express the relation of the specified food constituents, in grams.

The carbohydrate deprivation test probe's the individual's sensitiveness to ketogenic provocation. Ketosis is produced by fasting or by a diet, the K./A. ratio of which is well above the average limit of ketogenesis. The ketosis which develops is observed clinically and tested in the laboratory by repeated analyses of blood and urine, and hypoglycemia and ketonuria can thus be determined both as to time of onset and as to intensity. The decrease in the respiratory quotient can be observed at the same time.

PEDIATRIC CONSIDERATIONS

Children do not react like adults to withdrawal of carbohydrates. These physiologic differences must be kept in mind when children are tested. As a rule, they are more sensitive to ketogenic provocation (41), and ketonuria appears at K./A. ratios of less than 0.7 or F.A./G. ratios of 1 or less (40). In adults, the ketogenic borderline has been found at ratios of 1 and 1.5, respectively.

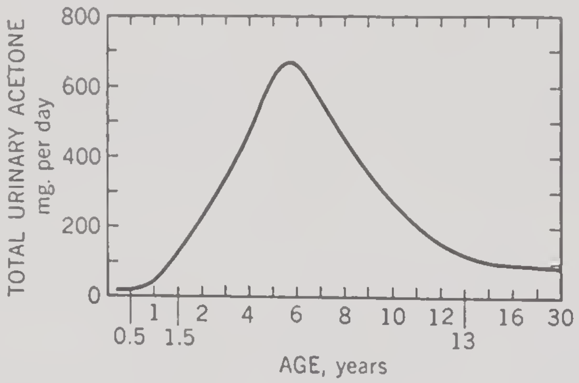

Fig. 23. Variation with age in disposition to ketosis. Approximate relation between age and degree of ketonuria induced by ketogenic diet (K./A. ratio, 2.5), on the basis of Heymann's (42) observations on 36 normal persons between the ages of 2 months and 35 years. Ketone bodies are expressed in terms of total acetone.

The metabolic response to carbohydrate deprivation also is not uniform throughout childhood. In infants, hypoglycemia is the first and most conspicuous result, and ketonuria is absent or negligible. Progressively with age the hypoglycemic tendency declines and the disposition to ketonuria increases. Heymann (42) believes that the ability to develop ketosis begins at the age of 7 to 8 months, reaches a broad peak during the age of 4 to 8 years, and drops toward the adult level during prepuberty. However, the level still remains higher than during infancy (Fig. 23). Obviously, then, a child's response to the test must be interpreted on the basis of its disposition to hypoglycemia and ketonuria with respect to its age.

The clinical usefulness of the test in young children is limited by two factors: (*1*) the physiologic occurrence of extreme sensitivity to carbohydrate deprivation; and (*2*) the widely varying reactions of children in the same age group. No attempt should be made, therefore, to classify results as normal or pathologic; the only possible differentiation is between weak and strong responses. No specific diagnosis can be made from a child's reaction to the test; positive results simply point to a disposition to change from carbohydrate oxidation to fat oxidation. However, in the presence of a definite clinical picture, a specific diagnosis may be supported. When, for example, a test meal brings on ketonuria and vomiting in a child with a history of recurrent vomiting, good supporting evidence is provided for a diagnosis of cyclic vomiting.

PROCEDURE

For the purposes of this test, a subject may be deprived of carbohydrates either by an enforced period of fasting or by ingestion of a high fat–low carbohydrate diet. For children up to the age of 2 years fasting is the better method, one or more feedings being omitted to achieve a fast of 8 to 24 hours. In children over the age of 2, only prolonged periods of fasting will produce ketosis, and a

TABLE 31
Guide for Selecting Ketogenic Diets

Age, years	Weight, Kg.	Water	Protein	Carbohydrate	Fat, Gm.
		Gm. per Kg. body weight			
Preschool	Up to 19	30–40	2	0.7	$\frac{60 \times \text{Kg. body weight}}{9}$
5–10	20–32	20–30	1.5	0.5	$\frac{50 \times \text{Kg. body weight}}{9}$
Over 10	Over 32	15–25	1.5	0.4	$\frac{40 \times \text{Kg. body weight}}{9}$

From McQuarrie (43).

high fat–low carbohydrate diet is therefore the better method for them.

The ketogenic diet is started abruptly, in contrast to the method recommended for the treatment of epilepsy. Diets with a K./A. ratio of 2 to 2.5 are usually selected. McQuarrie's scheme (Table 31) may be used for calculating the required amounts of protein, fat, and carbohydrate, and one of the commonly used food tables, e.g., Sherman's (44), for the selection of foodstuffs for such a diet.

Knöpfelmacher (45) suggests a diet which may be given any child between the ages of 3 and 8 years. It contains 1,830 Calories, 68 Gm. protein, and 40 Gm. carbohydrate; quantities can be varied according to the child's age. With slight modifications, the diet is as follows:

8 a.m.: 250 Gm. water, or weak tea with saccharin, 20 Gm. bread, 50 Gm. cheese, 20 Gm. butter.
12 noon: 300 Gm. broth with 20 Gm. farina, 2 Gm. fat, 4 Gm. flour, 250 Gm. vegetables with 50 Gm. fat.
4 p.m.: 250 Gm. water, or weak tea with saccharin, 1 cracker with 50 Gm. cheese and 10 Gm. butter.
7 p.m.: 50 Gm. fried meat, 200 Gm. vegetables with 30 Gm. fat.

Heymann (46) obtained prompt ketogenic action, for the purposes of this test, by giving a vegetable diet composed of 300 Gm. broth, 3 eggs, 50 Gm. fat, 300 Gm. vegetables, and tea with saccharin.

Urine is collected at 2 to 4 hour intervals for as long as the diet is maintained, and examined for ketone bodies and sugar. Blood is examined for sugar (page 99) immediately before the diet is started and every 6 hours thereafter.

The child must be carefully observed during the entire test period, day and night. The clinical response not only represents one of the criteria by which the test is evaluated, but may also reveal the onset of acute hypoglycemic and acetonemic reactions (see below). When these appear, the test must be stopped immediately, and 10 per cent glucose must be administered intravenously at once, as well as 0.5 mg. epinephrine subcutaneously.

When the fasting test is performed on infants, it is terminated in less than 24 hours, whether there is a response or not; in most older children the test should be continued until a metabolic reaction is elicited. On the average, this takes about 36 hours. This test, too, should be stopped as soon as ketonuria and/or hypoglycemia become intense, and glucose administered.

One of the commonly used clinical tests for ketonuria is the sodium nitroprusside test as modified by Rothera (47), which permits a rough estimate of the degree of acetonuria.

Reagents.

(*1*) Nitroprusside reagent. Mix 5 Gm. dry, powdered sodium nitroprusside with 200 Gm. ammonium sulfate.

(*2*) Commercial concentrated aqueous ammonia.

Sufficient nitroprusside reagent is added to 2 to 3 cc. of urine in a test tube to saturate it. The saturated urine is well shaken and then overlayed with 0.5 to 1 cc. of aqueous ammonia. If ketone bodies are present, a purple color develops in the upper portion of the tube's contents, i.e., in the zone penetrated by the ammonia, reaching maximum intensity within 2 to 3 minutes and then changing to a muddy brown. Even faint traces of acetone will produce a purple track through the white foam. The appearance of a violet-pink to purple-blue color is interpreted as a positive reaction (1 to 2 plus), while a deep, dark purple indicates the presence of abundant amounts (3 plus) of acetone bodies.

The presence of salicylates in the urine will also produce a positive reaction. To rule out such false positive results, a urine sample is diluted with an equal volume of water, boiled down to its original volume, and cooled. If the reaction of this sample is as strongly positive as that of the untreated urine, the color is due to the presence of drugs.

INTERPRETATION

Reactions are classified either as weak or moderate, or as strong. For all practical purposes, negative reactions can be disregarded. In the mild to moderate response, hypoglycemia and ketonuria develop at a slow rate, becoming noticeable only after 15 or more hours of the ketogenic regimen. After a period of adjustment, metabolism is stabilized on the acidotic level and the child remains comfortable and shows no abnormal clinical signs. Yet analyses of blood and urine, and the respiratory quotient, all reveal the slow but steady rise of ketosis. This slow type of reaction, observed in the majority of healthy children (Figs. 24, 25), indicates marked tolerance for ketogenic provocation.

The early appearance of severe ketosis, a rapid fall of the blood sugar to 50 mg. per hundred cubic centimeters or less, a sharp drop in the respiratory quotient, and clinical signs such as abdominal distress, nausea, vomiting, restlessness, dizziness, apathy, somnolence, convulsions, are characteristic of a strong reaction to the test. All these signs and symptoms may become manifest as early as 8 to 10 hours after the start of the test. A common feature of severe reactions is the considerable loss of weight, due to acidotic dehydration (Fig. 24). Strongly positive reactions, a sign of high susceptibility to, or low tolerance for, ketogenic provocation are found in

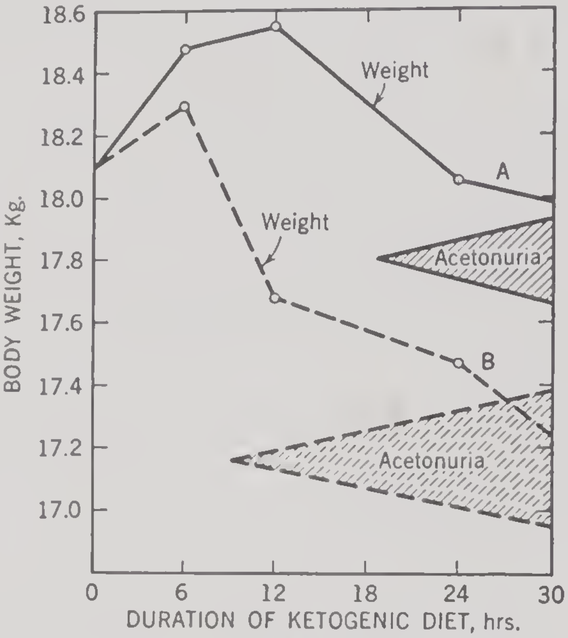

Fig. 24. Carbohydrate deprivation test. Rate of acetonuria and loss of body weight during ketogenic diet. A: Normal 7 year old child. B: 7 year old child with cyclic vomiting, tested during free interval. From Heymann (46).

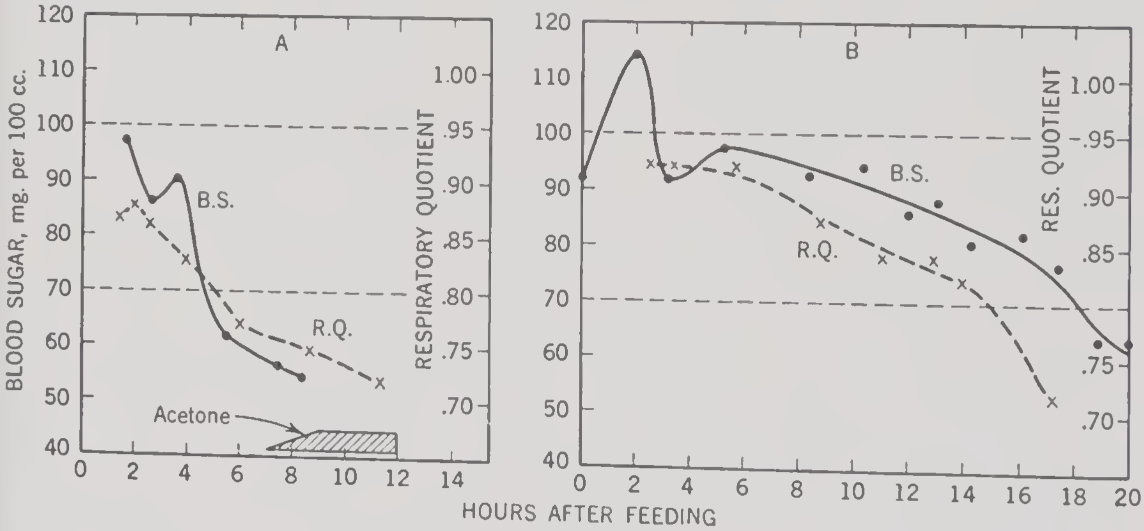

Fig. 25. Carbohydrate deprivation test. Rate of fall in blood sugar and respiratory quotient during fasting. A: 15 month old child with glycogen disease, approximately same weight as child in B. B: 6 month old normal infant, weighing 7.2 kg. From Bridge and Holt (48).

(*1*) a small number of healthy children 3 to 8 years old, (*2*) a small group of neurasthenic children, (*3*) numerous cases of cyclic vomiting (Fig. 24), (*4*) almost all children suffering from acetonemic convulsions; (*5*) all diabetic children, (*6*) a small number of children with hepatogenic hypoglycemia (glycogen storage disease) (Fig. 25), and (*7*) children with hypofunction of the adrenal cortex.

In cyclic vomiting and acetonemic convulsions these strong reactions can be provoked during the intervals between acetonemic attacks, when no clinical signs of the disorders are manifest.

High sensitiveness to carbohydrate deprivation is frequently associated with abnormally increased susceptibility to adrenalin (page 125) and insulin (page 120).

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CHAPTER VI

Protein Metabolism Tests

Proteins are primarily structural substances, or in Quick's term (1) "functional structures." With the phospholipids they form the structural basis of cells, framing the space that harbors the processes of life, such as metabolism and the exchange of gases. Structural proteins participate only to a very limited extent in intracellular biologic processes, nor are proteins of any great importance as a source of energy. Except in the liver, proteins are stored only in limited quantities in the organs and tissues of the body. According to Whipple (2), liver cells can store a reserve of plasma protein-forming material which may amount to one to five times the circulating mass. This storage, and the release of fabricated protein into the circulation, are essential factors in maintaining protein balance, which explains why the protein content of plasma and erythrocytes has such important physiologic implications.

The synthesis of proteins in tissues is accomplished by the utilization of amino acids; these are derived from the proteins in the food, and are supplied to the tissues by the blood stream. Some amino acids are used to form purine compounds and creatine. The physiologic degradation of tissue proteins is accomplished by hydrolysis of the protein molecules into the amino acid "stones" of which they are composed, the chief end product of this process being urea and ammonia. Table 32 gives the nitrogenous substances which are normal constituents of blood and urine.

While there has been remarkable progress in elucidating the chemical phases of protein synthesis and catabolism, development of tests to show the course of synthesis and catabolic hydrolysis of body proteins in man has been comparatively slow. There are tests to determine the balance between the formation and disintegration of tissue proteins; directly, by determining the nitrogen balance, and indirectly, by analyzing the total plasma proteins, which are closely related to tissue proteins. By both methods protein depletion may be disclosed. The results of another group of tests, consisting of the fractional analysis of plasma proteins and examination of blood and urine for the end products of protein metabolism, divulge the chemical pathology of intermediary reactions and their probable sites. Finally, there are tests for tolerance to proteins and their derivatives. The aim of such tests is to assess a subject's capacity to utilize one or another protein constituent that is administered orally or parenterally.

TABLE 32
Nitrogenous Substances Normally Found in Blood and Urine

In blood		In urine
Protein N	Nonprotein N	Nonprotein N
Hemoglobin	Urea	Urea
Albumin	Uric acid	Ammonia
Globulin	Creatine	Uric acid
Fibrinogen	Creatinine	Creatinine
	Amino acids	Undetermined (amino acids, hippuric acid, purines, allantoin)
	Undetermined (ammonia, purines, unknown substances)	

Only a small number of the many tests comprised in the three groups are useful for practical diagnostic purposes. Measurements of the nitrogen balance, for example, are complex procedures and require the facilities of a metabolic ward for many days, and tolerance tests with amino acids are still in the experimental stage. Determination of plasma proteins, therefore, remains the most practicable procedure for routine examination of the efficiency of an individual's protein metabolism. Further feasible clinical tests are those for creatine and creatinine excretion and tolerance. The functional significance of these tests is enhanced by the fact that creatine and creatinine are compounds which take part in the transformation of chemical into physical energy.

No elements of the nonprotein nitrogen in the blood and urine, except for creatine and creatinine, will be discussed in this chapter, despite the fact that these substances (aside from uric acid) are end products of protein metabolism. Their concentration in blood and urine is conditioned by renal function rather than by the state of protein metabolism. Assay of blood nonprotein nitrogen is outlined on page 161; of urea in blood and urine, on page 371.

Neither functional nor pediatric considerations demand a discussion of proteinuria at this point. A test method for albuminuria may be found on page 481.

DETERMINATION OF PROTEIN FRACTIONS IN PLASMA

Fibrinogen, albumin, and globulin are the three main protein fractions found in human plasma. They are most probably produced in the liver; albumin and globulin may also originate in the spleen and bone marrow, although this has not yet been definitely established (2). Fibrinogen is primarily concerned in the process of blood clotting, while the most important function of serum albumin and globulin is the maintenance of osmotic pressure and of the acid-base balance in the blood.

Chemical and physicochemical methods are available for the determination of plasma proteins. Microrefractometric methods (3) permit estimation of albumin and globulin percentages in very small quantities (0.5–1.5 cc.) of serum. For those who still prefer this method—its reliability has been disputed—an improvement in calculating the refractive index has recently been recommended (4).

Electrophoretic analysis is the newest and probably the most sensitive method of determining the distribution of protein fractions in plasma. The electrophoretic pattern reveals changes in globulin and albumin fractions at an earlier stage than is possible with any other method (5a).

The "density gradient tube" method has now been adapted for the determination of total serum proteins (5b). The procedure seems almost ideally suited for routine use in testing children, particularly for large-scale studies of protein nutrition, because only 2 or 3 c.mm. of serum are required.

Chemical determination of plasma protein is based on the separation of the three fractions by the "salting out" process, and measurement of the nitrogen content of each fraction. If plasma is used, three separate samples are needed for analysis for fibrinogen, albumin, and total protein. If serum is used, two samples are needed

—one for albumin and the other for total protein. The fractions which are not determined analytically can be computed.

Many analytic methods have been recommended which are based on these principles. Micromodifications have steadily reduced the amount of blood needed. For example, the method described by Rapoport *et al.* (6) calls for only 0.2 cc. of serum for the estimation of albumin and globulin.

The procedure of Hill and Trevorrow (7), described below, is especially designed for use in children, and can easily be substituted for macromethods commonly used in the clinical laboratory. It requires 0.2 cc. of plasma or serum for albumin and globulin determinations, and no more than 0.4 cc. of plasma for the complete analysis for protein fractions. Tests for plasma proteins in infants and young children no longer need be omitted merely because the laboratory requests a larger quantity of blood than can be obtained in a particular child.

PROCEDURE

The method described is that of Hill and Trevorrow (7).

Reagents.

(*1*) 10 per cent solution of sodium tungstate.

(*2*) $^2/_3$ N sulfuric acid.

(*3*) Buffer solution. Dissolve 10.05 Gm. potassium dihydrogen phosphate and 508 cc. of 0.1 N sodium hydroxide in water to make 1 liter.

(*4*) Fibrinogen precipitant. Dissolve 104.6 Gm. sodium sulfate in buffer solution to make 1 liter.

(*5*) Globulin precipitant. Dissolve 218.5 Gm. anhydrous sodium sulfate in buffer solution to make 1 liter. Both precipitants (*4* and *5*) may be hazy at first and develop a flocculant precipitate, but this does not interfere with the test.

(*6*) Digestion mixture. Combine 3 volumes concentrated sulfuric acid and 1 volume "syrupy" phosphoric acid.

(*7*) 30 per cent hydrogen peroxide.

(*8*) Indicator. Mix 1.25 Gm. methyl red, 0.825 Gm. methylene blue, and 90 per cent ethyl alcohol to make 1 liter.

(*9*) Nessler's solution (commercially available).

(*10*) Stock ammonium sulfate solution. Dissolve 9.4332 Gm.

ammonium sulfate in water and dilute with 0.2 N sulfuric acid to make 1 liter.

(*11*) Standard nitrogen solution. Add 20 cc. stock ammonium sulfate solution to 180 cc. of 0.2 N sulfuric acid and dilute to 1 liter with water. The standard contains 0.04 mg. nitrogen per cubic centimeter.

If the test is to be performed with plasma, 1–2 cc. of whole blood are needed; if with serum, 2–4 cc. of whole blood. Coagulation is prevented by adding 0.5 mg. heparin.

Determination of Total Nitrogen. 0.1 cc. of plasma or serum is diluted with 1 cc. of water in a 25 × 100 mm. pyrex tube, and 1 cc. of digestion mixture is added.

Determination of Nonprotein Nitrogen (N.P.N.). 0.1 cc. of plasma or serum is diluted with 1 cc. of water in a 15 cc. tapered centrifuge tube, and 0.1 cc. of 10 per cent sodium tungstate and 0.1 cc. of $^2/_3$ N sulfuric acid are added. The mixture is stirred, centrifuged for 15 minutes, and the clear supernatant fluid is transferred by means of a capillary tube to a test tube. From it, 0.7 cc. is transferred into a 25 × 200 mm. pyrex tube and 1 cc. of digestion mixture is added.

Determination of Fibrinogen Nitrogen. 0.1 cc. of plasma is added to 1.9 cc. of the fibrinogen precipitant in a 15 cc. tapered centrifuge tube, the contents are mixed, and the tube is allowed to stand for 3 hours. The tube is then centrifuged for 10 minutes, the supernatant fluid is poured off, and the tube is inverted over filter paper to drain. Then 1.9 cc. of fibrinogen precipitant are again added to the tube, the contents are mixed, and centrifugation, decantation, and draining repeated as before. The remaining fibrinogen pellet is transferred quantitatively with 2–5 cc. of water to a 25 × 200 mm. pyrex tube and 1 cc. of the digestion mixture is added.

Determination of Albumin Nitrogen + Nonprotein Nitrogen. 0.1 cc. of plasma or serum is added to 1.9 cc. of globulin precipitant in a 15 cc. tapered pyrex centrifuge tube, the contents are mixed, and allowed to stand for 3 hours at room temperature. The tube is then centrifuged for 45 minutes, and the supernatant fluid, which is the albumin solution, is transferred to a test tube.

If marked lipemia is present, centrifugation is omitted and the tube is allowed to stand for 24 hours. The mixture is then filtered through a small funnel 1–1.5 cm. in diameter, the filter paper having been moistened with 95 per cent alcohol and dried in a current of air. Filtration is repeated until the albumin solution is clear. To prevent evaporation, filtration may be done in a large desiccator containing globulin precipitant to a depth of 2.5 cm.

1 cc. of the albumin solution, obtained either by centrifugation or filtration, is pipetted into a 25 × 200 mm. test tube and 1 cc. of digestion mixture is added.

Digestion. The four fractions are now ready for digestion and estimation of nitrogen as ammonium. An "ammonia blank" is run with each fraction, containing water instead of plasma or serum. To each tube, 0.6 cc. of the digestion mixture and 2 glass beads are added. One at a time, each tube is carefully heated over a microburner until the contents give off dense fumes. The tube is allowed to cool for a minute, 2 drops of 30 per cent hydrogen peroxide are added, and the tube heated again until fumes fill the tube. If the brown color of the solution has not disappeared, hydrogen peroxide is added again and heating repeated. The clear solution is cooled and 10 cc. of water are added. Finally, sodium hydroxide, a little more than is necessary to neutralize the acid mixture, is added to each tube. Each of the four fractions and blanks are digested in this manner.

Distillation. Ammonia from the first and fourth samples, containing total nitrogen and albumin nitrogen, respectively, is distilled with a micro-Kjeldahl apparatus into exactly 1 cc. of 0.1 *N* sulfuric acid contained in a receiving test tube of 20–30 cc. capacity. Pregl's modification (8), which employs steam for the distillation of ammonia, is the preferable technic. The distillation is completed when the volume of the mixture has been reduced by half. Two drops of the indicator are added to the receiving tube, and the excess of sulfuric acid is titrated with 0.1 *N* sodium hydroxide.

Ammonia from the second and third samples, containing nonprotein nitrogen and fibrinogen nitrogen, respectively, are distilled into an empty test tube graduated at 21 and 30 cc. When distillation is completed, as above, the distillate is diluted with water to 21 cc. and the tube is placed in a water bath at 25 C. Into a similar tube are transferred 3 cc. of standard nitrogen solution and water to the 21 cc. mark, 9 cc. of Nessler's solution are added to both standard and unknown, and the tubes are returned to the water bath. The tubes are read in a visual colorimeter within the following 15 minutes. If the nitrogen content of the distillate exceeds that of the standard, colorimetric determination is performed on an appropriate aliquot of the distillate. By multiplying by 1.2 the quotient of the reading of standard over reading of unknown, one obtains the number of milligrams of nitrogen in the distillate.

Calculation.

Total protein:

$$\text{cc. } 0.1\ N\ H_2SO_4 - \text{cc. } 0.1\ N\ \text{NaOH} - \text{blank} = \text{cc. } 0.1\ N\ NH_3 \text{ distilled}$$

$$\frac{\text{cc. } 0.1\ N\ NH_3 \text{ distilled} \times 1.4 \times 100}{\text{volume analyzed plasma}} = \text{mg. total N/100 cc.}$$

$$\frac{\text{mg. total N} - \text{N.P.N.}}{1{,}000} \times 6.25 = \text{Gm. protein/100 cc.}$$

Nonprotein nitrogen: If not analyzed, it is assumed to amount to 25 mg. per hundred cubic centimeters. If analyzed:

$$\frac{(\text{mg. N in disuillate} - \text{blank}) \times 1.3 \times 100}{\text{volume analyzed plasma} \times 0.7} = \text{mg. N.P.N./100 cc.}$$

Fibrinogen:

$$\frac{(\text{mg. N in distillate} - \text{blank}) \times 1.3 \times 100 \times 6.25}{\text{volume analyzed plasma} \times 0.7 \times 1{,}000} = \text{Gm. fibrinogen/100 cc.}$$

Albumin:

$$\text{cc. } 0.1\ N\ H_2SO_4 - \text{cc. } 0.1\ N\ \text{NaOH} - \text{blank} = \text{cc. } 0.1\ N\ NH_3 \text{ distilled}$$

$$\frac{\text{cc. } 0.1\ N\ NH_3 \text{ distilled} \times 2.8 \times 100}{\text{volume analyzed plasma}} = \text{mg. N/100 cc.}$$

$$\frac{\text{mg. N/100 cc.} - \text{mg. N.P.N./100 cc.}}{1{,}000} \times 6.25 = \text{Gm. albumin/100 cc.}$$

Globulin:

In plasma:

$$\text{Gm. protein/100 cc.} - \text{Gm. albumin/100 cc.} - \text{Gm. fibrinogen/100 cc.} = \text{Gm. globulin/100 cc.}$$

In serum:

$$\text{Gm. protein/100 cc.} - \text{Gm. albumin/100 cc.} = \text{Gm. globulin/100 cc.}$$

$$\text{Albumin/globulin ratio} = \frac{\text{Gm. albumin/100 cc.}}{\text{Gm. globulin/100 cc.}}$$

Correction should always be made for the nonprotein nitrogen in the samples analyzed for albumin and total protein. If there is any indication of nitrogen retention due to renal insufficiency, the nonprotein nitrogen must be determined analytically in a separate sample of plasma.

INTERPRETATION

While a number of different "normal values" are to be found in the literature on plasma proteins in children (9), the differences are partly the result of the different analytic methods used. The normal concentration of plasma proteins in the various age groups, as determined by the authors of the micromethod just described, supplemented by the findings of other workers, is given in Table 32A. These figures, representing the means of normal values, vary widely. From the table it can be seen that the general tendency of the plasma proteins is to increase with age, and that by the age of 5 the normal adult levels are reached. More specifically, changes in the normal concentration of the various fractions are as follows: (*1*) Total protein, which is 5.7 Gm. per hundred cubic centimeters in the newborn, rises slowly but consistently through infancy and early childhood, reaching the adult level toward the end of the fifth year. (*2*) There is no significant change with age in the mean fibrinogen value from the age of 2 months to 5 years. During the first month, a tendency toward a higher mean value is apparent. There is a wide scatter of values throughout childhood. (*3*) Albumin increases with age, subject to seasonal variations. (*4*) Globulin varies with age, the lowest concentration occurring between the

TABLE 32A
Normal Mean Values of Protein Fractions in Plasma*

Age	Total protein	Fibrinogen	Albumin	Globulin	A./G.
	Grams per 100 cc.				
Newborn, premature (10)	4.9	—	—	—	—
Newborn, full term (11)	5.7	0.26	3.73 (10)	1.66	2.0
1 mo. (11)	5.33	0.26	3.79	1.31	
2–6 mos. (11)	│	0.21	4.28 (10)	1.31	
6–12 mos. (11)	↓	↓	4.7	↓	
4 yrs. (11)	6.94	0.21	4.7	2.3	
Adults (11)	6.94	0.25 (12)	4.7	2.03	

* Numbers in parentheses are reference numbers.

second and sixth months of life. (*5*) The albumin–globulin ratio is above 2 throughout childhood, except during the first 6 months when the values are around 3.

Dehydration, particularly in children, will result in "false normal values." Anhydremia may raise the protein level by 50 per cent, thereby obscuring an initial hypoproteinemia. In all cases of presumed dehydration, therefore, hematocrit determinations are essential.

Hypoproteinemia, indicating protein deficiency, may be the result of: (*1*) failure of synthesis, due to impaired liver function; (*2*) inadequate intake, due to nutritional deficiencies; (*3*) loss, as in hemorrhage, burns, severe albuminuria or uncontrolled diabetes; and (*4*) increased demand, due to a high basal metabolism, or to an abnormally increased requirement for dietary protein, when fat and sugar assimilation are at fault. Depletion of albumin and a corresponding fall in the albumin–globulin ratio will almost always be the chief feature of hypoproteinemia. However, the other two fractions may participate in the depletion and thereby influence both the total protein concentration and the albumin–globulin ratio.

Conditions often associated with hypoproteinemia which are of concern to the pediatrician show the following characteristic changes in the partition of plasma proteins: (*1*) after hemorrhage all three fractions may be low as a result of blood dilution; the albumin–globulin ratio, however, remains essentially normal. (*2*) Depletion of albumin also characterizes the hypoproteinemia which occurs during postoperative states (13). (*3*) In severe liver diseases, albumin is depleted while the globulin fraction is unchanged or even increased. The extent of the change may vary, but it always results in a characteristic lowering of the ratio to about 1. When the ratio falls below 1, it is termed "reversal of ratio"; in the absence of Bright's disease, such a reversal is considered pathognomonic of severe impairment of hepatic function (13). These pathologic changes are most often seen in hepatic cirrhosis (14); they are less common and less pronounced in parenchymatous liver disease (5a). The occurrence in children of interstitial hepatitis with continued hypoproteinemia and demonstrable liver atrophy (15) should be mentioned here. (*4*) In nephrosis and nutritional edema, the hypoproteinemia is mainly the result of a reduction in albumin, the globulin fraction remaining unchanged or decreasing only slightly.

Total protein values, therefore, fall to 3 to 5 Gm. per hundred cubic centimeters or less, depending on the child's age. The albumin–globulin ratio is usually below 1.5, and may fall as low as 0.3. Edema commonly appears when the albumin drops to less than 2.5 Gm. per hundred cubic centimeters.

The characteristic changes just described, and the promising results recently obtained in the treatment of nephrosis with concentrated, low salt, human serum albumin (16,17), are clearly demonstrated by the course which the levels of the protein fractions follow during long periods before and after the start of treatment.

Fibrinogen deficiency is almost invariably the result of extreme liver damage (18). It occasionally occurs as a congenital deficiency (19a) which has been termed "pseudohemophilia" (19b). Acquired fibrinogen deficiency is the result of cirrhotic changes in the liver which reach an advanced stage, such as in thrombosis of hepatic vessels and in Banti's disease. Decrease and increase in fibrinogen are not dependent on the other plasma protein fractions (11).

Hyperproteinemia, in the absence of dehydration, is almost invariably the result of an increase in globulin. It is commonly associated with extensive structural liver damage, or diseases involving the bone marrow, particularly with myeloma (20). Severe infections may have a similar effect. When the hyperproteinemia is of hepatic origin, it is a sign of an advanced stage of the same functional impairment which in the earlier phases leads only to a decrease of plasma albumin and a corresponding change in the albumin-globulin ratio. Tests which demonstrate qualitative changes in the composition of plasma globulin are the Hanger test, the colloidal gold test, the Takata-Ara test, and the thymol turbidity reaction (pages 43–49).

Hyperproteinemia is an important factor in dehydration. The concomitant percentual increase in hemoglobin concentration and the rise in hematocrit value indicate the presence of anhydremia. The albumin–globulin ratio is not altered significantly. In mild forms, the total plasma protein does not exceed 8 Gm. per hundred cubic centimeters; in extremely dehydrated infants, the concentration may rise above 8 Gm. and reach 10 Gm. per hundred cubic centimeters. However, in a child with depleted plasma proteins dehydration may cause the protein level to rise to a seemingly normal percentual value, although it is actually reduced (page 165).

AMINO ACID TOLERANCE TESTS

First used by Folin and Berglund (21a), these tests are based on the common principle of loading tests, namely, determination of the blood level curve after administration of a test substance, which in this instance is protein or protein derivatives. The results of the oral tolerance tests may show: (*1*) the fate of amino acids in the intermediary metabolism, provided their intestinal absorption has been normal, and (*2*) the efficiency of the digestive proteolysis of proteins, and the intestinal absorption of its end products, provided their subsequent intermediary assimilation is normal. Tolerance curves after intravenous injection of amino acid mixtures throw a light on the metabolic utilization of amino acids, a function mainly dependent on hepatic efficiency, and perhaps on the avidity of the tissues for amino acids.

In the main, the tests have proved to be of little value in testing ability to dispose of absorbed protein products; nor do they serve as a criterion of liver function (21b). However, the postprandial curve of blood amino acids has been successfully used to demonstrate faulty digestion of proteins due to absence of pancreatic enzymes in the duodenal juice (21c). The development of micromethods for estimating amino acids in small samples of blood, such as may be obtained serially from small children, has greatly aided the use of such tests in children.

The analytic procedure consists of the assay of amino acid nitrogen in the blood. The most accurate results are obtained with the gasometric ninhydrin–carbon dioxide method of Hamilton and Van Slyke (21d,22), which calls for 0.5 cc. of plasma for the single determination. Frame, Russel, and Wilhelmi (23) recommend a simpler colorimetric method for ordinary clinical purposes, although it is not equally accurate. This procedure requires 0.2 cc. of blood for a double analysis. West *et al.* (21e) have recently described an adaptation of the colorimetric method for use with the Evelyn photoelectrometer.

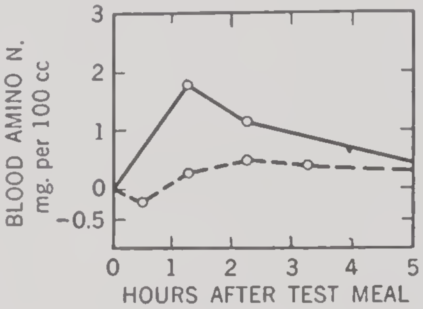
The normal range of the blood amino acid nitrogen level is approximately the same in adults and children—from 3 to 5.5 mg. nitrogen per 100 cc. of plasma (24,25), or from 4.5 to 8 mg. nitrogen per 100 cc. of blood (25). The fasting level of amino acid nitrogen in the blood is of little diagnostic significance, except in the nephrotic syndrome, in which a chronic deficit of amino acid nitrogen has been found throughout the duration of the disease (24); the values range from 3 to 4 mg. nitrogen per 100 cc. of plasma. A further sharp drop, below 2.5 mg., occurs during acute nephrotic crises in young children (24). These findings are in agreement with the belief expressed by Farr and MacFadyen (24) that "the failure of children with the nephrotic type of Bright's disease to regenerate plasma proteins . . . cannot be attributed solely to loss of protein in the urine in all cases," but are in part attributable to metabolic disorders involving the intermediary metabolism of protein. During the acute phase of infectious diseases, particularly pneumonia, the decreased level of amino acids in plasma suggests an impairment of protein synthesis due to toxic products (25).

Oral Test. A single dose of protein or protein derivates is given by mouth after an overnight fast, and the curve of the blood amino acid nitrogen is observed for the following 5 hours. Since the nature and dose of the protein substances containing the amino acids, as used by the various investigators, has not been uniform, standards for the normal response to the test can be given only by referring them to the kind of test meal ingested. Generally, about 2 Gm. of protein per kilogram of body weight suffice to bring about a good rise of the blood amino acid level in normal adults. In a recent study (21c), West *et al.* secured data from normal children, using approximately the same dosage. For young infants these investigators used a test feeding composed of a mixture of 1.7 Gm. casec (calcium caseinate), containing 1.5 Gm. protein, and 30 cc. water per kilogram of body weight. For larger infants and children, water amounting roughly to 1½ times the volume of the dry casec was added. Infants must be given their test meal by gavage. Blood specimens were taken before, and ½, 1¼, 2¼, and 5 hours after ingestion of the test meal. Capillary blood is preferable; it should be collected directly from a heel or finger tip puncture into a pipet calibrated to deliver 0.2 or 0.5 cc., depending on the analytic method to be used.

Normally in response to the protein test dose, the amino acid nitrogen curve rises ½ hour after ingestion of the test meal. In many cases, the ½ hour level represents the greatest rise; occasionally, the highest point of the curve is reached 1¼ hours after ingestion; it is rarely later. In every case, the amino acid nitrogen in the 1¼ hour specimen is 1 mg. or more above the fasting level (Fig. 25a).

If the curve fails to show this normal rise, the most probable reason is that the amount of amino acids produced by the enzymic proteolysis in the intestines and absorbed into the blood is too small to raise the blood nitrogen. Such flat curves have been observed in children with cystic fibrosis of the pancreas (21c) as a result of the lack of pancreatic trypsin in the duodenal juice (Fig. 25a). These abnormal curves show a general tendency to remain parallel to the fasting level throughout the 5 hour period, or to show a rise toward the end of the observation period. In contrast to such results, patients with celiac disease respond to the test normally, since their pancreatic trypsin activity is intact.

Fig. 25a. Oral amino acid tolerance test. Amino nitrogen absorption curves following ingestion of casein. The values are reduced to a zero fasting level. Upper curve: Normal, 1 week old infant. Lower curve: 5 month old infant with cystic fibrosis of pancreas. From West, Wilson, and Eyles (21c).



When the test is performed with test meals composed of protein hydrolysates, which contain preformed amino acids, the response in both celiac disease and cystic fibrosis of the pancreas is of the normal type; no essential proteolytic activity is required, and the ingested amino acids are absorbed normally. If the postprandial blood amino acid curve is flat after a test meal of protein hydrolysate, the cause must be sought in the abnormal tissue avidity for amino acids, which may cause amino acids to disappear from the blood stream with unusual rapidity.

An abnormally high and extended rise of the curve may indicate not only efficient intestinal absorption but also impaired utilization of amino acids, whatever the test meal. In such instances, hepatic insufficiency may be the cause, as explained below.

Intravenous Test. Lyttle and co-workers have studied (26) the curves following intravenous loading tests in children, using a 10 per cent solution of casein hydrolysate (commercially available) as a test substance. A cubic centimeter of this solution represents about 6 mg. of amino acid nitrogen. With the child in a fasting state, 2 cc. of hydrolysate per kilogram of body weight, diluted with an equal volume of 5 per cent glucose in saline, are injected intravenously within a period of 4 to 5 minutes. The maximum dose for older children is 40 cc. of hydrolysate, diluted with 40 cc. of 5 per cent glucose in saline. Blood samples are taken before and 5, 15, 35, and 95 minutes after the injection is completed, and the amino acid nitrogen in each sample is estimated by the ninhydrin method.

In infants and children up to the age of 10 years the high plasma level of amino acids normally declines sharply within the first 5 to 10 minutes after the injection and returns to the initial levels after 35 to 95 minutes. If the return to the fasting level is delayed beyond 95 minutes after injection, it is considered as evidence of pathologic retention of amino acids. Such abnormal curves have been obtained in patients with portal cirrhosis or other severe hepatic disease. It it believed that delayed clearing of the loading dose of amino acids is indicative of extensive hepatic insufficiency, provided renal function is intact. The curves obtained in nephrotic children after intravenous loading are essentially normal.

URINARY CREATINE–CREATININE RATIO

Most of the creatine (methyl-guanidine-acetic acid) in the human body is found in muscle; 80 per cent of it is combined with phosphoric acid as phosphocreatine. Creatinine, the anhydride of creatine, is present in muscle in relatively small amounts.

It is believed that muscle is the site of formation of both creatine and creatinine. Creatine is synthesized from amino acids, such as arginine, glycine, and methionine, but the origin of creatinine is still open to question. Usually it is referred to as a waste product of the nitrogen metabolism of muscle; however, the view that it is derived principally from the creatine in muscle is not generally accepted.

In adults, creatine is present in blood in small amounts, but is normally absent from the urine, except occasionally in women. Creatinine, however, is a normal constituent both of blood and urine throughout life. For technical and other reasons, creatine and creatinine metabolism has been studied by investigating their urinary excretion rather than their blood levels, and the normal ranges of creatine and creatinine output are fairly well established for all age groups. Both the actual amount of creatine and of its anhydride in a 24 hour urine specimen and the creatine–creatinine ratio are of clinical significance. However, the results of such studies have no diagnostic significance in states of renal insufficiency with retention in the blood of nonprotein nitrogenous substances, including creatine.

PEDIATRIC CONSIDERATIONS

The metabolism of creatine and creatinine in children shows some physiologic peculiarities. Unlike adults, children normally excrete creatine in the urine, since the growing organism lacks the ability to store creatine derived from ingested food. This considerable creatine output gradually diminishes with age, and ceases at about the age of puberty (27). The excretion of creatinine, however, which is commonly considered an index of the amount of active protoplasmic tissue, shows the opposite trend; it increases steadily during childhood. These reciprocal changes with age are the reason why the urinary creatine–creatinine ratio varies only between 20/100 and 40/100 from infancy through puberty. In studies on children, the creatine and creatinine actually excreted during 24 hours should be referred to body weight, expressed in kilograms, since such "coefficients" represent a more biologic approach to the evaluation of actual values.

Urinary creatine and creatinine assays are helpful diagnostic aids in differentiating neuromuscular disorders, such as dystonias and dystrophies. Abnormal excretions of creatine and creatinine may also be caused by pathology of the thyroid gland, and the rate of excretion is a very reliable standard of reference for evaluating basal heat production in children (page 75).

PROCEDURE

The child to be tested should be on a diet normal for his age. Urine should be collected accurately and completely for a 24 hour period; the specimens are pooled in a stoppered bottle and covered with 5 to 10 cc. of 5 per cent thymol in chloroform as a preservative. The total volume is measured and recorded. Aliquots are taken out for analysis.

The analytic method described here is that of Folin (28) as modified by Reiner (29).

Reagents.

(*1*) Saturated picric acid solution. Add about 2 Gm. picric acid to 100 cc. of water. Filter just before use.

(*2*) 10 per cent sodium hydroxide.

(*3*) Standard creatinine solution. Dissolve 100 mg. creatinine in 100 cc. of 0.1 *N* hydrochloric acid.

Specimen 1, for "preformed creatinine": 2 cc. of urine are pipetted into a 100 cc. volumetric flask. Specimen 2, for "total creatinine": 2 cc. of urine are transferred into a 250 cc. Erlenmeyer flask, 20 cc. of the picric acid solution and an antibump tube* are added, and the flask with its contents is weighed. Then 150 cc. of water are added and the contents are boiled over a small flame until flask and contents are again about the original weight. The antibump tube is rinsed off with water and removed, and the flask is allowed to cool.

To make up the standards, 1 and 2 cc. of the creatinine standard solution are transferred into two 100 cc. volumetric flasks. To each flask, and to the flask containing specimen 1, 20 cc. of the picric acid solution are added. Then 1.5 cc. of the 10 per cent sodium hydroxide are added to the 3 volumetric flasks and the Erlenmeyer flask. After 10 minutes, specimen 2, contained in the Erlenmeyer flask, is transferred quantitatively to a 100 cc. volumetric flask, and water is added to volume to all 4 flasks. Specimens 1 and 2 are then compared with the standards in a colorimeter.

Calculation.

Mg. creatinine in 24-hr. vol. urine =

$$\frac{\text{standard}}{\text{unknown}} \times \text{mg. creatinine in standard} \times \frac{\text{24-hr. vol. urine}}{\text{cc. urine analyzed}}$$

Specimen 1 gives the amount of preformed creatinine; specimen 2 gives the amount of total creatinine, i.e., preformed creatinine plus hydrolyzed creatine. Creatine, expressed as creatinine, is calculated as the difference between specimens 1 and 2.

$$\text{Creatinine coefficient} = \frac{\text{mg. creatinine/24-hr. urine}}{\text{Kg. body weight}}$$

$$\text{Creatine coefficient} = \frac{\text{mg. creatine/24-hr. urine, expressed as creatinine}}{\text{Kg. body weight}}$$

Total creatinine coefficient = creatinine coefficient + creatine coefficient

It should be noted that there is another form of coefficient in use, which gives, as numerators, milligrams of creatinine nitrogen and creatine nitrogen instead of milligrams of creatinine and creatine, as above. The formula for conversion is as follows:

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* Seal ends of 180 mm. length of 4 mm. tubing; seal 15 mm. length of same tubing to one end of the long tube, and fire polish open end.

$$\text{Mg. creatinine N} = \text{mg. creatinine} \times 0.372$$

These coefficients should be clearly designated as nitrogen coefficients, and they are calculated as follows:

$$\text{Creatinine N coefficient} = \frac{\text{mg. creatinine N/24-hr. urine}}{\text{Kg. body weight}}$$

$$\text{Creatine N coefficient} = \frac{\text{mg. creatine N/24-hr. urine, expressed as creatinine N}}{\text{Kg. body weight}}$$

Total creatinine N coefficient = sum of creatinine N and creatine N coefficients

Example. Child, age 8 months, weight 6.55 Kg. By analysis, performed creatinine (specimen 1) equalled 91.86 mg., total creatinine (specimen 2) equalled 165.09 mg. Creatine, i.e., the difference between specimens 2 and 1, equalled 73.23 mg.

Creatinine coefficient: $91.86/6.55 = 14.02$
Creatine coefficient: $73.23/6.55 = 11.18$
Total creatinine coefficient: $14.02 + 11.18 = 25.2$
Creatinine N coefficient: $(91.86 \times 0.372)/6.55 = 5.22$
Creatine N coefficient: $(73.23 \times 0.372)/6.55 = 4.16$
Total creatinine N coefficient: $5.22 + 4.16 = 9.38$

INTERPRETATION

Creatine excretion gradually diminishes with age. Although data for the newborn infant are "almost nonexistent" (30), the high rate of excretion—about 9 mg. per kilogram of body weight in 24 hours—in infants 1 month old and over has been definitely established (27). As the child grows older, excretion gradually decreases, falling to about 2 mg. per kilogram by the age of 14 to 15 years; during pubescence it ceases completely. The normal range fluctuates moderately above and below the average values, as given in Table 33.

When the deviation from these averages is considerable and constant, the creatine output should be considered abnormal. Tables 34 and 35 show, respectively, the clinical significance of abnormal creatine excretion and the amounts of urinary creatine actually found in various conditions.

The normal range of *creatinine* output gradually increases from about 5 to 9 mg. per kilogram of body weight at the age of 2 weeks to about 20 mg. at the age of 15 years. From Table 33 it may be seen that except in early infancy the physiologic excretion of creatinine and creatine shows a reciprocal relationship.

TABLE 33

Average Normal Urinary Creatine and Creatinine Output

Age*	Creatine coeff.	Creatinine coeff.	Total creatinine coeff.	Creatine N coeff.	Creatinine N coeff.	Total Creatinine N coeff.
Full term infant, 2nd week (33)	—	5-9	—	—	1.9-3.3	—
1-7 mos. (27)	8.9	12.8	21.7	3.3	4.8	8.1
2-3 yrs.	7.9	12.1	20.0	3.0	4.5	7.5
4-4½ yrs.	4.5	14.6	19.1	1.7	5.4	7.1
9-9½ yrs.	2.5	18.1	20.6	1.0	6.7	7.7
11-14 yrs.	2.7	20.2	22.9	1.0	7.5	8.5
Adult man	0	19-27	19-27	0	7-10	7-10

Adapted from Harding and Gaebler (31,32).
* Numbers in parentheses are reference numbers.

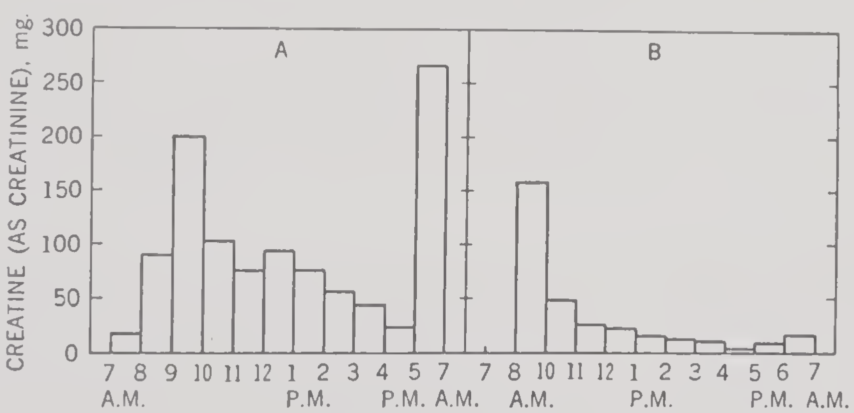

Fig. 25b. Creatine tolerance test. Hourly urinary excretion of creatine after ingestion of 1 Gm. creatine at 8 A.M. A: 11 year old boy with progressive muscular dystrophy; 24 hour excretion on test day was 959.9 mg., on control day, 264.2 mg. B: 9 year old normal boy; 24 hour excretion on test day was 324.2 mg., on control day, 37.5 mg. After Magee (34).

Abnormal creatinine output is found in relatively few clinical instances (Table 34). There is a marked decrease in progressive muscular dystrophy, sometimes to 50 per cent of the normal average (Table 35). So far as we know, there are no reports of abnormally increased creatinine excretion in children.

Normally, total creatinine is remarkably constant throughout childhood, if the values are referred to body weight (Table 33). It is approximately the same as the adult output of preformed creatinine, which is identical with the adult value for total creatinine.

TABLE 34
Changes in Urinary Creatine and Creatinine Output in Certain Pathologic Conditions during Childhood

Condition	Creatine	Creatinine	Total creatinine
Prematurity	Absent or reduced	Reduced	Reduced
Hypothyroidism	Absent or reduced	Normal	Reduced
Hyperthyroidism	Increased	Normal	Increased
Starvation	Increased	Normal	Increased
Myotonia congenita	Reduced	Normal	Reduced
Myasthenia gravis	Increased	Normal or reduced	Reduced
Progressive muscular dystrophy	Increased	Reduced	Reduced

TABLE 35
Urinary Creatine and Creatinine Excretion in Pathologic Conditions

Condition*	Age	Coefficients in terms of mg. creatinine N/24 hrs./Kg. for		
		Creatine	Creatinine	Total creatinine
Prematurity (27)	3–4 wks.	0.3–1.8	4.4–5.5	4.8–7.1
Progressive muscular dystrophy (34)	11 yrs.	4.0	2.0	6.0
Hypothyroidism (35)				
Untreated	14 mos.	0.04	5.3	5.34
After 6 weeks' treatment	15 mos.	3.5	5.7	9.2
Untreated	5 yrs.	0	7.9	7.9
After 6 weeks' treatment	5 yrs.	5.2	6.3	11.5

* Numbers in parentheses are reference numbers.

Abnormal creatine or creatinine excretion results in abnormal values for total creatinine. Instances of such unusual values for total creatinine, due to abnormal creatine–creatinine ratios, are given in Table 35.

CREATINE TOLERANCE TEST

The test, as devised by Shorr (36), consists of oral administration of a known quantity of creatine to a patient on a creatine-free diet, and subsequent urinalysis for creatine. The amount of creatine excreted reflects the individual's ability to retain and metabolize creatine. If this ability is impaired, an abnormally large portion of the test dose will be excreted, with a concomitant decrease in urinary creatinine. The significance of the test is conditioned by the renal clearance of creatine.

PEDIATRIC CONSIDERATIONS

The reaction of children to ingestion of creatine is governed by the physiologic inability of the growing organism to store creatine. Since the protein and creatine content of the child's diet exceeds his tolerance and leads to continuous creatinuria, the only possible reaction to the strain of additional creatine is an increase in the physiologic creatinuria.

The test in children, therefore, leads to an increase in the existing creatinuria and excretion of a larger portion of the ingested test dose than in adults. Opinion as to the clinical usefulness of the test in children is divided. Fairly constant results are reported in some studies, supporting the belief that typical differences exist between the normal low tolerance and the extremely low tolerance associated with certain diseases. Other results, however, make the usefulness of the test seem doubtful (35).

PROCEDURE

The directions outlined by Magee (34) and Sovahl, King, and Reiner (37), are as follows: Infants and young children are fed their normal milk diet; all other children are kept on a purine-free diet (no meat or meat products, fish, fowl, soups, cocoa, or chocolate) for 3 days. On the fourth or control day, collection of urine is begun at 7 A.M., and is continued for 24 hours, and for 2 additional periods of 24 hours each. A 10 per cent solution of thymol in chloroform serves as preservative. The test dose of creatine hydrate is given by mouth at 7 A.M., on the fifth day. Dosage is 40 to 60 mg. per kilogram of body weight for children weighing up to 5 Kg., 30 to 40 mg. per kilogram for children weighing 5 to 20 Kg., 10 to 20 mg. per kilogram for children of 20 to 30 Kg. weight, and the standard dose of 1.32 Gm. for children over 30 Kg. in weight. For small children the creatine is dissolved in the regular feeding mixture; for older children, in 180 cc. of water. The three 24 hour urine specimens are analyzed for creatine by the method described on page 171.

Calculation. The amount of creatine excreted during the 24 or 48 hour period following ingestion of the test dose minus the amount excreted on the control day gives the amount of "extra creatine" derived from the ingested test dose. The extra creatine, expressed as creatinine, is reported as per cent of ingested creatine, 1.32 Gm. creatine hydrate being equivalent to 1 Gm. urinary creatine, expressed as creatinine.

INTERPRETATION

Healthy infants, according to Marples and Levine (27), excrete 55 to 65 per cent of the ingested dose in the first 24 hours, and a total of 63 to 82 per cent in the first 48 hours. The average is 60.7 and 72.9 per cent, respectively. The existing data for children from 2 to 12 years of age are conflicting, and are insufficient to form a basis for standard values.

The excretion in children over 12 years of age may be assumed to be about the same as in adults, namely, 30 per cent or less of the ingested creatine in the first 24 hours (38).

If creatine excretion after a test dose is greater than the normal figures for the age, the presence of an abnormally low creatine tolerance is indicated. Complete recovery of the test dose in the 48 hour period has been reported in cases of amyotonia congenita, progressive muscular atrophy, myositis fibrosa, and severe hyperthyroidism (39). Evidence of inability to retain exogenous creatine is particularly valuable in the diagnosis of progressive muscular dystrophy (page 395).

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CHAPTER VII

Inorganic Body Constituents

CALCIUM AND PHOSPHORUS

The alimentary tract is the place where calcium, magnesium, and phosphorus are brought into a state most suitable for resorption. According to Schmidt and Greenberg (1) resorption is chiefly promoted by two agencies operating in the intestines—the enzymes and the hydrogen ions. The enzymes split phosphoric acid from the ester compounds, while the hydrogen ions keep calcium phosphate in solution. The acid phosphates are more soluble and can be absorbed more easily than the basic salts of phosphoric acid. Calcium is largely absorbed in the upper parts of the small intestine, where the acid gastric juice is still incompletely neutralized and where calcium exists mainly in the form of chloride and acid phosphate. Phosphorus, on the other hand, is absorbed in considerable amounts only after it reaches the lower parts of the small intestine, where the rate of enzymic cleavage of ester compounds is highest.

In the healthy individual the intestines are the chief site not only for calcium and phosphorus absorption but for their excretion as well. Part of the fecal calcium and phosphorus is derived from unabsorbed food, the other part is endogenous and excreted by the intestine.

Once absorbed, calcium and phosphorus travel by way of the blood to the tissues, where they serve to form anatomic structures and maintain metabolic processes. There is good evidence in support of the view that vitamin D plays a role in the proper retention of both elements, although the mechanism of its action is not yet clearly understood. Vitamin D is also considered a prerequisite for the absorption of calcium, magnesium, and phosphorus (1).

The tissues are constantly releasing calcium and phosphorus back into the blood stream, thus compensating for the loss via the urine and feces. The parathyroids control the rates of mobilization and excretion, and the level of the two minerals in the blood is kept almost rigidly constant. Disorders of calcium and phosphorus metabolism may involve absorption, transportation, utilization, and excretion.

Many procedures have been devised for testing the calcium and phosphorus nutrition and the various phases of their metabolism, yet the tests which can be used clinically are few. So far, the only reliable method of assessing their assimilation and catabolism is to study the calcium and phosphorus balance. The biologic significance of such studies has been greatly enhanced by the use of radioactive isotopes, which now make it possible to demonstrate "the fluidity of the chemical constituents of the body and to discover pathways and mechanisms" of assimilation and catabolism (2). For clinical use, however, these studies are too laborious, requiring food, urine, and stool analysis for at least 3 days, in addition to control periods.

Although efforts to devise function tests have in general not been successful, two of these proposed methods—the calcium excretion test and the phosphate tolerance test—will be described here. So far they have been rarely applied to children. Analyses of blood and urine for calcium and phosphorus therefore remain virtually the only procedures in common use in pediatric practice.

Total Calcium in Serum or Plasma

Blood calcium is found only in plasma, and according to McLean and Hastings (3) its concentration in plasma is one of the physiologic constants. The calcium content of erythrocytes is negligible. An abnormal plasma calcium level may be the result either of an abnormal rate of calcium mobilization or of an abnormal rate of excretion. The concentration of total calcium in serum is likewise affected by changes in serum concentrations of phosphates and proteins.

Serum may be analyzed for total calcium by colorimetric, volumetric (titrimetric), or gasometric procedures, the volumetric being the most commonly used. The easiest of the volumetric procedures are those which do not require removal of proteins as a preliminary to the precipitation of calcium. Clinical laboratories, for this reason, prefer Kramer and Tisdall's method, which requires 2 cc. of serum or plasma. But for children methods requiring a smaller amount of serum are to be preferred.

Total proteins and inorganic phosphate in serum should be routinely determined, along with total calcium.

PEDIATRIC CONSIDERATIONS

The choice of methods for use in children may be facilitated by the following list arranged according to quantity of serum needed for a single determination.

(*1*) With 2 cc. of serum: Titrimetric estimation by Kramer and Tisdall's method, as modified by Clark and Collip (4). The precipitated calcium oxalate is titrated with potassium permanganate.

(*2*) With 1 cc. of serum: (*a*) Colorimetric determination by the method of Roe and Kahn (5). Calcium is precipitated as tricalcium phosphate and the phosphate is measured by the method of Youngburg and Youngburg (6). (*b*) Gasometric analysis according to Van Slyke and Sendroy (7). The precipitated calcium oxalate is redissolved and the solution is shaken with potassium permanganate. The carbon dioxide evolved is measured in the Van Slyke–Neill apparatus.

(*3*) With 0.2 cc. of serum: (*a*) The manometric wet combustion method of Van Slyke and Kreysa (8). Calcium is precipitated as picrolonate, and the precipitate is estimated from the carbon. This method calls for much technical detail and special apparatus. (*b*) The photometric determination of Kuttner and Cohen, as modified by Youngburg and Youngburg (6,9,10). Calcium is precipitated as tricalcium phosphate and the phosphorus is measured. (*c*) The iodometric titration of Rappaport and Rappaport, as modified by Biering (11). Calcium is precipitated by oxalate, the washed precipitate is dissolved, and cerisulfate is added. The amount of cerisulfate not reduced to cerosulfate is determined by iodometric titration.

(*4*) With 0.1 cc. of serum: Titrimetric estimation by the method of Sobel and Sobel (12). The calcium oxalate precipitate is converted to the carbonate and the calcium is estimated by direct acidimetric titration after addition of boric acid.

The most convenient methods for serial determinations are the ones using 0.1 or 0.2 cc. of serum. A detailed description follows of three of the methods listed above: (*1*), (*3b*), (*4*).

In interpreting analytic results, the accepted standards for adults may be used, since the difference between normal figures for infants and adults is so slight that no essential diagnostic error can occur, except in the newborn.

PROCEDURES

ANALYSIS FOR TOTAL SERUM CALCIUM BY KRAMER AND TISDALL'S METHOD AS MODIFIED BY CLARK AND COLLIP (4)

Apparatus. Microburet; centrifuge tubes graduated in 0.02 or 0.05 cc., with a fine tip and with the capillary end measuring about 5 mm. in length and 2 mm. in diameter (available commercially).

Reagents.

(*1*) Ammonium water. Add 2 cc. concentrated ammonia to 98 cc. water.

(*2*) 4 per cent ammonium oxalate.

(*3*) Potassium permanganate stock solution (approximately 0.1 *N*). Dissolve 3.16 Gm. potassium permanganate in 900 cc. distilled water, allow to stand for 1 week, then filter through glass wool, dilute 1 liter, and store in a brown bottle.

(*4*) 0.01 *N* potassium permanganate solution. Dilute 100 cc. stock solution with distilled water to 1 liter and store in brown bottle.

(*5*) 0.01 *N* sodium oxalate solution. Dissolve 0.67 Gm. sodium oxalate, dried at 100 C. for 12 hours, in distilled water, add 5 cc. concentrated sulfuric acid, dilute to 1 liter, mix well, add a few drops of toluol as preservative.

Determine the titer of the 0.01 *N* potassium permanganate solution whenever it is to be used by titrating against 1 cc. of the 0.01 *N* sodium oxalate solution to which 2 cc. of *N* sulfuric acid have been added.

Technic. Into a centrifuge tube are measured 2 cc. of serum, or plasma from citrated or heparinized blood, 2 cc. of water, and 1 cc. of 4 per cent ammonium oxalate solution, the contents are thoroughly mixed, allowed to stand for 30 or more minutes, and then centrifuged until the precipitate is well packed in the bottom of the tube. The supernatant liquid is carefully poured off, and while still inverted the tube is placed in a rack for 5 minutes to drain,* with the mouth of the tube resting on a pad of filter paper. The mouth of the tube is then wiped dry with a soft cloth, the precipitate is stirred up, and the sides of the tube are washed with approximately 3 cc. of dilute ammonia water directed from a wash bottle in a very fine stream. The suspension is again centrifuged and drained as before, and 2 cc. of normal sulfuric acid are blown directly from a pipet upon the precipitate so as to break up the mat and facilitate solution. The tube is then placed in a boiling water bath for about a minute, after which the contents are titrated with 0.01 *N* potassium permanganate, using a micropipet. The end point is reached when a definite pink color persists for at least 1 minute.

Titration is best carried out in a water bath at a temperature of 70 to 75 C. The water bath consists of an 800 cc. pyrex beaker filled with water and heated over a flame or by an electric heating device in the water. The temperature should be constantly controlled, by a thermometer if a flame is used, by a rheostat if the heating is by electric device.

A blank, consisting of 2 cc. normal sulfuric acid and 1 cc. water, is treated similarly.

Calculation. With a 2 cc. sample of serum,

$$\text{Mg. calcium per 100 cc. serum} = (U - B) \times F \times 10$$

where U is cc. 0.01 N potassium permanganate used in titration of sample and B is cc. 0.01 N potassium permanganate solution used in titration of blank.

$$F = 1/(O - B)$$

where O is cc. 0.01 N potassium permanganate required to titrate 1 cc. of 0.01 N sodium oxalate (titer) and B is cc. 0.01 N potassium permanganate solution used in titrating the blank.

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* To insure uniform drainage, the tubes should always be cleaned before use with cleaning solution by heating at approximately 100 C. for a few minutes.

ANALYSIS FOR TOTAL SERUM CALCIUM BY YOUNGBURG AND YOUNGBURG'S METHOD (6,10)

Apparatus.

(*1*) Electrocolorimeter.

(*2*) Narrow test tubes (12 × 120 mm.), calibrated at 1 and 2 cc.

Reagents.

(*1*) 7 per cent trichloroacetic acid, with 0.5 cc. syrupy phosphoric acid per liter.

(*2*) Alkaline sodium phosphate mixture. Dissolve 1 Gm. basic trisodium phosphate in 50 cc. distilled water and mix with 50 cc. of 20 per cent sodium hydroxide. If a precipitate forms, allow to settle for 24 hours or centrifuge a small portion for immediate use.

(*3*) Alkalinized alcohol. Dilute 55 cc. of 95 per cent alcohol with water, make faintly alkaline with calcium-free sodium hydroxide, using litmus paper as indicator, and make up to 100.

(*4*) Molybdic–sulfuric acid mixture. Pour 282 cc. concentrated sulfuric acid (sp. gr. 1.84) into 600 cc. distilled water, cool, transfer to a one-liter flask, and make up to volume with distilled water. Dissolve 7.5 Gm. sodium molybdate in distilled water in a 100 cc. graduated flask, and make up to volume with distilled water. For use, dilute 1 volume of the sulfuric acid solution with 2 volumes of distilled water, allow to cool if necessary, and then pour into 1 volume of the sodium molybdate solution.

(*5*) Stannous chloride stock solution. Dissolve 10 Gm. stannous chloride in 25 cc. concentrated hydrochloric acid. Store in a cool place in a brown, glass-stoppered bottle. For use, dilute 0.5 cc. stock solution to 100 cc. with distilled water. This diluted reagent does not keep.

(*6*) Standard phosphate stock solution. Dissolve 0.4394 Gm. dried monopotassium phosphate in 1 liter distilled water and add a few drops of chloroform. 1 cc. of the solution is equivalent to 1 mg. phosphorus.

Technic. Serum or plasma, 0.2 cc., is transferred to a narrow calibrated test tube, 7% trichloroacetic acid is added to the 2 cc. mark, the tube is closed with a rubber stopper, shaken, and centrifuged for 5 minutes. Then 0.5 cc. of the clear, colorless supernatant fluid, equivalent to 0.05 cc. of the serum sample, is transferred to another narrow test tube, 0.2 cc. of the alkaline phosphate mixture is added, and the tube is set aside for 1 hour. It is then centrifuged for 3 minutes at high speed. The supernatant liquid is discarded, the last drop being caught on a piece of blotting paper, the tube is allowed to drain for 2 minutes, and the rim is then wiped with filter paper. The precipitate in the tube (difficult to see unless a colored indicator is used) is washed twice with 1 cc. portions of the alkalinized alcohol. After each washing and centrifuging the tube is drained and the rim is wiped, as above. Then 0.4 cc. of the molybdic–sulfuric acid reagent is added, the contents mixed by tapping the tube with a finger, and 0.5 cc. distilled water and 0.1 cc. diluted stannous chloride reagent are pipetted into the tube. The tube is immediately closed with a rubber stopper, and inverted; after 1 minute the colored solution is transferred into the absorption cell of the electrocolorimeter and the galvanometer reading is noted.

Cells of 2.5 cc. capacity and an orange filter must be used. If the minimum amount of liquid required for this set-up is more than 1 cc., the unknown solution is diluted with 0.5 or 1 cc. of distilled water before the tube is stoppered and inverted.

A blank is treated similarly.

Calculation. A calibration curve for phosphorus is prepared for the given quantitative conditions of the procedure described. In such a curve, the galvanometer readings are charted against the respective phosphorus concentrations, expressed in milligrams phosphorus per 100 cc. of serum, covering a range from 0.5 to 4 mg. of phosphorus, corresponding to 1 to 8 mg. phosphorus per 100 cc. of serum. Detailed directions for preparing calibration curves in general, and the phosphorus curves in particular, may be found in textbooks (47a) (see also page 206).

The milligrams of phosphorus per 100 cc. of serum which correspond to the galvanometer reading of the unknown sample is determined directly from the phosphorus calibration curve. In similar fashion, the phosphorus value which corresponds to the reading of the blank is determined.

The difference between the unknown and the blank represents the milligrams of inorganic phosphorus in 100 cc. of serum. This figure is multiplied by the factor 1.94 to obtain milligrams of calcium in 100 cc. of the serum analyzed.

ANALYSIS FOR TOTAL SERUM CALCIUM BY SOBEL AND SOBEL'S METHOD (12)*

Apparatus.

(*1*) Self-filling capillary microburet, containing mercury and equipped with a micrometer arrangement. A special type of microburet used by the authors of the method is commercially available.

(*2*) Specially designed centrifuge tubes. They are made of pyrex glass, have a wall thickness of 1.2–1.5 mm., and over-all length of 69–71 mm., and an internal diameter of 10–12 mm. The conical portion is about 23 mm. in length and has a flat bottom of approximately 2 mm. diameter. The flat bottom is important. The tube is rimmed so that it may be held in the rack attached to the microburet.

(*3*) 0.1 cc. capillary pipet graduated in 0.01 cc. The over-all length of the graduated portion is 140–150 mm.

(*4*) Fine aspirating pipet, drawn down from 7mm. tubing. To be used as aspirator, the pipet is attached to a water pump.

(*5*) Muffle furnace.

Reagents.

(*1*) Saturated solution of ammonium oxalate, prepared at 40–50 C. and allowed to cool to room temperature. The clear supernatant solution is used.

(*2*) 0.5 per cent ammonium oxalate.

(*3*) 10 per cent boric acid solution. Dissolve 10 Gm. boric acid in 100 cc. distilled water by heating. Use the solution while hot.

(*4*) Indicator. 5 parts of 1 per cent brom-cresol-green solution in 95 per cent alcohol is mixed with 1 part of 1 per cent methyl red solution in 95 per cent alcohol. For use, a diluted indicator is prepared by making up 6 drops of the mixed indicator to 5 cc. with distilled water.

Technic. Into 1 of the centrifuge tubes are measured 0.1 cc. serum or plasma, and 0.1 cc. saturated ammonium oxalate. The contents are mixed by tapping the tube gently, care being taken that the solution does not rise more than halfway up the tube, the tube is stoppered, and allowed to stand for 3 hours. The stopper is then removed and the tube is centrifuged at about 2,000 r.p.m. for 15 min. The supernatant fluid is aspirated with a fine pipet to within 1 mm. of the flat bottom of the tube. The precipitate and the liquid remaining in the tube are mixed by gentle tapping; 0.3 cc. of 0.5 per cent ammonium oxalate is added and mixed by gentle tapping, to wash the precipitate. Centrifugation for 15 minutes and aspiration of supernatant fluid to within 1 mm. of the bottom are repeated. The tube is then placed in an oven at 110 C. until the precipitate is dry, and then in a muffle furnace at 475 to 525 C. for 30 minutes; this converts the oxalate to carbonate. A sand bath may be used if a muffle furnace is not available. The tube is removed from the furnace or sand bath, allowed to cool, then immersed in a boiling water bath, and 0.05 cc. of hot 10 per cent boric acid solution is added. The precipitate dissolves completely in a few minutes. The tube is removed from the water bath, 0.2 cc. diluted indicator is added to the hot solution, and mixture aided by gentle tapping. When the solution has cooled to room temperature it is ready for titration.

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* Through the courtesy of Dr. Albert E. Sobel, the description includes some changes he has made in the original procedure.

A capillary microburet, the tip of which is immersed in the solution, and 0.01 *N* sulfuric acid or hydrochloric acid are used for the titration. The solution is titrated back to the *p*H of a pure boric acid solution of similar strength, a point reached when the color of the unknown solution has become identical with that of a boric acid solution prepared for comparison. This is done by pipetting 0.05 cc. of the 10 per cent boric acid solution into a tube like the one containing the unknown, and adding 0.25 cc. diluted indicator. A gentle stream of air is bubbled through the unknown solution during the titration, to make certain that the added acid mixes properly with the solution.

Calculation. The titration represents the titrimetric equivalent of calcium determined directly. 1 cc. of 0.01 *N* acid equals 0.2 mg. calcium. If 0.1 cc. of serum is used, then:

$$\text{mg. Ca per 100 cc. serum} = 200 \times \text{cc. } 0.01\ N \text{ acid used in titration}$$

INTERPRETATION

Normal values for total calcium in serum are:

Age	Calcium, mg./100 cc.
Newborn, days 1–7 (13)	7.5–13.9
Infants (14)	10.5–12.0
Children (14)	10.0–11.5
Adults (14)	9.5–10.5

Averages which may be derived from these normal values are without worth, since only deviations beyond the limits of the normal range are of clinical significance. Normal values of total calcium do not preclude the presence of an abnormal partition of calcium in serum (page 193).

Hypocalcemia is characterized by values that are one or more milligrams below the low normal limits, while in hypercalcemia the values are one or more milligrams above the upper normal limits (14).

McLean and Hastings (3) list the following values of total calcium in serum as typical in clinical conditions associated with disorders of calcium metabolism:

Elevated (12–16 mg./100 cc.)	Within or near normal range (8–11.5 mg./100 cc.)	Lowered (4–8.5 mg./100 cc.)
Hyperparathyroidism	Osteomalacia	Hypoparathyroidism
Hyperproteinemia (especially in multiple myeloma)	Paget's disease	Hypoproteinemia
Vitamin D overdosage	Senile osteoporosis	Hyperphosphatemia of nephritis and uremia
	Calcinosis universalis	Rickets and osteomalacia (severe cases)
	Tetany of alkalosis	Infantile tetany

Relation of Serum Calcium to Serum Protein. Since the changes in total serum calcium are in direct proportion to the serum protein level, determination of the latter should always supplement that of total serum calcium. This is particularly important when the changes found in the serum calcium level are small. For example, when hypocalcemia occurs in conjunction with hypoproteinemia, the decrease in calcium may be regarded as physiologic and does not imply an imbalance in calcium distribution (3); but the same degree of hypocalcemia without an accompanying protein deficiency is a sign of true calcium depletion. Only in the presence of a normal serum concentration of protein does the calcium level (normal, low, or high) reflect normocalcemia, hypocalcemia, or hypercalcemia. The concentration of calcium ions furnishes the clue as to whether

TABLE 35A

Calcium-Protein Relationship in Serum. Hypocalcemic Response to Hypoproteinemia

Total protein, mg./100 cc.	Total calcium, mg./100 cc.	Ca^{++}, mg./100 cc.*
Normal		
7.6	10.9	4.8
7.0	10.5	4.8
6.3	10.0	4.7
Nephrosis		
5.8	9.8	4.8
4.7	9.5	5.2
4.1	8.1	4.6
3.8	7.5	4.6
3.2	7.1	4.6

After Gutman and Gutman (15).

* Computed from the nomogram on page 195.

TABLE 35B

Level of Serum Calcium and Inorganic Phosphorus in Children in Rickets and Tetany

Condition*	Total calcium, mg./100 cc.	Inorganic phosphorus, mg./100 cc.
Uncomplicated, active rickets (17)	Normal, or low normal (9–10)	Reduced (1–4)
In premature infants (16)	Low normal (9–10), or reduced	Reduced (2–4), or normal
Rickets accompanying celiac disease (18)	Reduced (<8.5)	Normal
Renal hyperparathyroidism (renal rickets) (19)	Normal, or reduced (7.5–10)	Increased (5–7)
Rachitic tetany (20)	Reduced (<8.5)	Normal, or high (4–6.5); occasionally reduced (3–4)
Hyperventilation tetany (21,22)	Normal, or high normal (11–12)	Slightly reduced (3–4)
Pyloric obstruction (gastric tetany) (23)	Normal, or high normal (10–12)	High normal, or increased (5–7)
Hypoparathyroid tetany (24)	Reduced (<7.5)	Increased (>5)
Toni-Fanconi syndrome with low phosphorus rickets (25)	Reduced (8–10)	Reduced (1–3)

* Figures in parentheses in this column only are reference numbers.

the fall or rise in total calcium is to be regarded as a physiologic response to changes in the serum proteins or as a pathologic condition. Assay of calcium ions renders unnecessary other ways of making allowance for the effect of abnormal protein values on total calcium figures. A normal level of calcium ions is characteristic of the physiologic association of hypocalcemia with hypoproteinemia.

Relation of Serum Calcium to Serum Inorganic Phosphate. Cohn *et al.* (2) state: "Calcium and phosphorus manifest their interdependence most strongly in blood and bone, particularly through the Ca^{++} and PO_4^{--} concentrations." Unlike proteins, inorganic phosphates in serum cause true disturbances in the calcium state, if they interfere at all. For this reason, when changes in calcium concentration are associated with changes in inorganic phosphorus, a pathologic condition in the state of serum calcium is always indicated. Not so much the degree as the mere presence of hyperphosphatemia or hypophasphatemia reflects the genesis and significance of an abnormal calcium level. The diagnostic value of the calcium–phosphorus ratio or of their product (C × P) under normal and certain abnormal conditions is open to question. According to Eliot and Park (16): "Since it [the product] does not supply any information which is not at once apparent from the calcium and inorganic phosphorus levels in the blood serum and gives a false sense of security, its use as absolute indicator should be abandoned."

However, the diagnostic significance of serum calcium values is greatly enhanced by values for serum inorganic phosphorus obtained simultaneously. This becomes apparent from a glance at Table 35B, which lists the wide variations in serum calcium and inorganic phosphorus in various forms of rickets and tetany.

Calcium Ion Concentration in Serum

Calcium is present in plasma in several forms (Fig. 26). About half of the total serum calcium (4–5 mg./100 cc.) is bound to proteins and is not diffusible; the greater portion of the remaining half exists in the ionized state (Ca^{++}), while the rest is present in some complex combination, with other substances which are as yet not identified (14). The latter two portions are diffusible, passing freely through semipermeable membranes. The ionized fraction, i.e., the dissociated calcium, probably exists in the form of supersaturated solutions of calcium carbonate and calcium phosphate. The concentration of this calcium is of great importance, both physiologically and clinically; calcium bound to protein and nonprotein complexes loses, with the electric charges, certain of its chemical properties, and some of its physiologic activities (3).

Since the total calcium level may not reveal fluctuations in serum calcium partition, the fractional analysis of serum calcium is of particular interest, and several procedures have been proposed for estimating the ionized fraction of calcium.

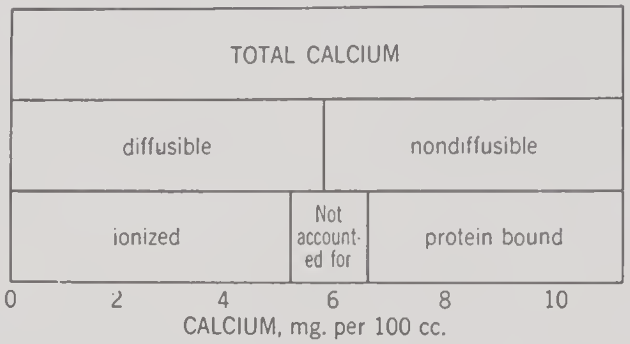

Fig. 26. Approximate, normal partition of calcium in human serum, if total serum protein is 7 per cent, and albumin–globulin ratio is 1.8. Chart is based on a report by McLean and Hastings (26) in terms of millimoles per kilogram water and converted by Gutman and Gutman (15) into milligrams per 100 cc. of serum.

There are first the direct electrometric measurements of calcium ions in body fluids. Second, the diffusible fraction may be estimated by dialysis and ultrafiltration. Greenberg and Gunther (27) describe a micromethod of ultrafiltration that is practicable for the clinical laboratory. If one holds that most of the filtrable calcium in the serum exists in the ionized form, the fraction obtained by ultrafiltration may be accepted as a rough estimate of calcium ionization.

Third, some workers (28,29) believe that the calcium ion concentration in serum can be estimated with fair accuracy by analyzing the cerebrospinal fluid for total calcium. This method is based on the theory that the fluid represents a dialyzate in which calcium exists almost exclusively in the ionized form.

Finally, the ionized calcium in serum can be calculated from the total calcium and protein in the serum. McLean and Hastings (3) have demonstrated that, at a given level of total calcium, the concentration of calcium ions varies in direct proportion to the amount of total protein in the serum.

The last-mentioned method, as recommended by McLean and Hastings, is now an important diagnostic aid. These authors (3) have shown that "if Ca^{++} concentrations are to remain within the normal range total calcium must rise and fall with changes in total protein and that such a change in the calcium level must be regarded as a normal physiologic response." On the other hand, in disorders which actually affect "calcium behavior," the calcium ion concentration is also affected, falling and rising in proportion to the total calcium, according to the mass law equation. Computation of calcium ion concentration by the McLean-Hastings formula or nomogram (Fig. 27) permits differentiation between the physiologic and pathologic geneses of abnormal total calcium levels.

PEDIATRIC CONSIDERATIONS

If assay of serum calcium ions is accepted as a means of interpreting changes in total calcium, there is ample opportunity to make use of this method in pediatric practice. In children with hypoproteinemia or hyperproteinemia measurement of calcium ions rather than of total calcium reveals the actual state of calcium distribution in the serum, while in other patients it may explain the apparent discrepancy between clinical findings and laboratory determination of total calcium.

The calcium ion concentration is the best index of a normal or abnormal protein–calcium ratio in serum. If determination of serum protein is routinely made with every determination of serum calcium, estimation of calcium ions automatically becomes part of the routine procedure for determination of serum calcium. The method, however, is subject to some limitations: (*1*) The McLean-Hastings formula, like other equations relating total calcium to total serum protein, does not apply when hyperproteinemia is present (15). (*2*) Changes in calcium ion concentration due to alkalosis or hyperphosphatemia are not revealed, and since hyperphosphatemia is physiologically present throughout childhood, calcium ion concentrations established by computation may, at least theoretically, be inaccurate. (*3*) In the newborn, the calcium ion concentration, as computed from the McLean-Hastings formula, is inconsistent with figures obtained by direct measurement of the filtrable fraction (30a).

PROCEDURE

To calculate calcium ions in serum by the McLean-Hastings method (3), serum is analyzed for total calcium (page 182) and total protein (page 160), and the value for calcium ions in milligrams per hundred cubic centimeters, is read from the nomogram (Fig. 27) as follows (3): "The point of intersection of a vertical line representing total protein concentration with a horizontal line representing total calcium concentration will fall on or between diagonal lines representing Ca++ concentrations, and its location

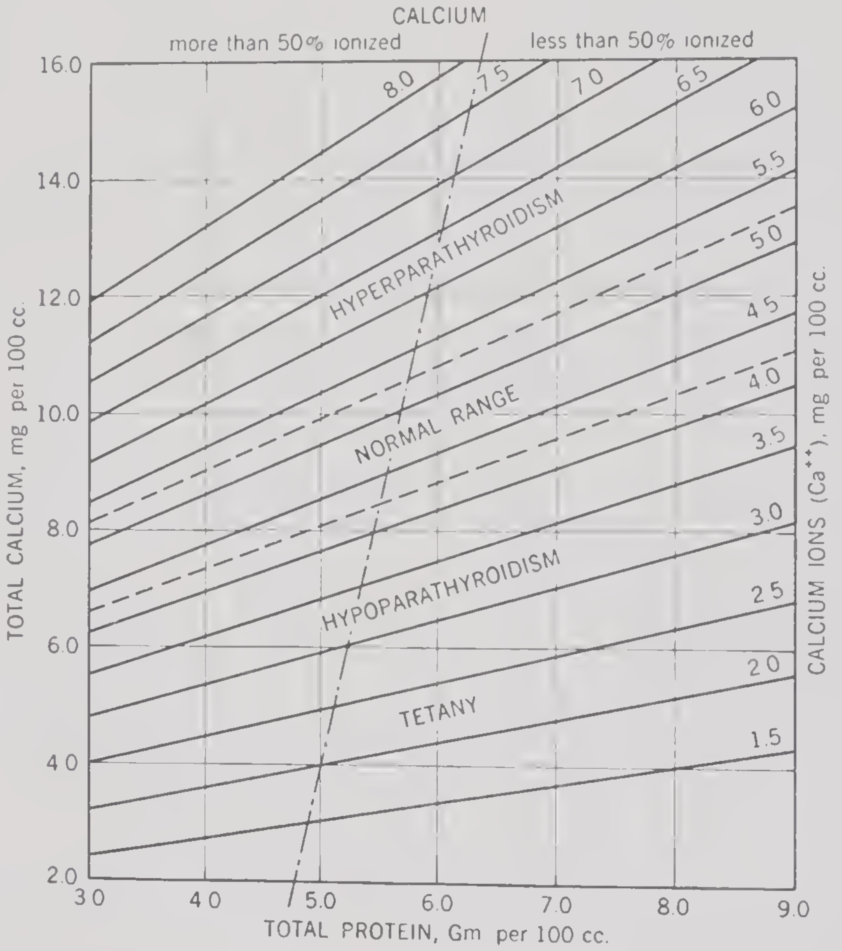

Fig. 27. Nomogram for calculating serum calcium ion concentration from total protein and total calcium content of serum. From McLean and Hastings (3).

with reference to these lines give at once the Ca^{++} concentration for the given condition."

The additional line dividing the chart into a left and right section includes all points at which calcium ions and calcium bound to protein are equal to each other, and serves as a guide to the proportion of calcium which is ionized under average conditions.

Calcium bound to protein equals total calcium minus ionized calcium.

INTERPRETATION

The normal range of calcium ion concentration in serum in adults and in children, as established by this method, is from 4.25 to 5.25 mg. calcium per hundred cubic centimeters (30b). These values agree well with the normal ionized calcium values, determined as the "diffusible" fraction, in children (31).

The normal value of calcium ion concentration in the newborn, calculated by the nomogram, is somewhat higher, running from 5.7 to 6.1 milligrams per hundred cubic centimeters (30b). But the level of "diffusible" calcium has not been found to be higher in the newborn than in older infants and children (30a). One must therefore conclude that the true state of calcium partition in the newborn is still not fully known.

Levels below 3.5 mg. per hundred cubic centimeters are usually associated with active tetany, such as occurs in rickets. Herlitz (30b), using the nomogram, arrived at the following values: 3.2, 2.8, and 1.5 mg. per hundred cubic centimeters in active rachitic tetany, and 3.8 and 4.8 mg. in latent rachitic tetany.

Values above 5.5 mg. per hundred cubic centimeters may be caused by hyperparathyroidism or by overdosage with vitamin D or dihydrotachysterol.

When the values for total calcium and/or total protein are abnormal, the nomogram is the method of choice for measuring the ionized fraction of calcium in the serum. In addition to Table 35A, the following examples illustrate the clinical significance of the value of calcium ion concentration thus obtained:

(*a*) The total serum calcium, by analysis, is 7.1 mg. per hundred cubic centimeters, a lower than normal value; total serum protein, by analysis, is 3.2 mg., also a lower than normal value; ionized Ca, computed by nomogram is 4.3 mg., a value within the normal range. The conclusion from these figures is that the fall in total calcium is a normal response to the change in serum protein. The "hypocalcemia" is without clinical significance, since the ionized calcium is at a normal level.

(*b*) The total serum calcium, by analysis, is 6 mg. per hundred cubic centimeters, an abnormally low value; total serum protein, by analysis, is 4.1 mg., also a lower than normal value; ionized Ca, computed by nomogram, is 3.3 mg., a lower than normal value also. The total calcium has dropped to a level too low to be accounted for by the hypoproteinemia, as indicated by the decrease in ionized calcium. This strongly suggests a disordered calcium distribution, i.e., hypoparathyroidism or hyperphosphatemia.

(*c*) The total serum calcium, by analysis, is 8.5 mg. per hundred cubic centimeters; a moderately low normal level; total serum protein, by analysis, is 7.2 mg., a normal level; ionized Ca, computed by nomogram, is 3.7 mg., a lower than normal value. The decrease in the ionized calcium is directly proportional to that in the total calcium, while the protein level has remained normal. These figures seem to indicate a true abnormality in the calcium metabolism; inorganic phosphorus concentration should be ascertained.

Whether the calcium–protein ratio remains constant when protein levels are higher than normal is not yet definitely established. The nomogram, therefore, should not be used in conditions associated with hyperproteinemia.

Obviously, when total calcium and protein levels are normal, the calcium ion concentration, as indicated by the nomogram, must also be normal. Potential changes in the ionized calcium fraction due to variables other than the protein concentration can then be revealed only by one of the other methods, preferably the microultrafiltration method. It is known that hyperphosphatemia and alkalosis often reduce the ionized calcium without altering total calcium concentration (normocalcemic forms of tetany) (32).

Urinary Excretion of Calcium

Normally when the calcium balance is in equilibrium or positive, the urinary calcium accounts for a smaller part of the total calcium excretion than the fecal calcium (68a). The renal threshold for calcium excretion lies between 6.5 and 8.5 mg. per 100 cc. of serum (33). While determination of both urinary and fecal calcium excretion is necessary for studies of calcium balance, the figures on urinary calcium alone are valuable in the early diagnosis of hyperparathyroidism and renal calculi. Hyperfunctioning parathyroids reverse the normal urinary calcium–fecal calcium ratio, 70 to 90 per cent being eliminated in the urine and only 10 to 30 per cent in the stools (34).

The semiquantitative Sulkowitch test (35), which reveals the presence or absence of excessive urinary excretion of calcium, may be used as a rough measure of calcinuria. To measure urinary excretion of calcium exactly, the patient must be kept under known dietetic conditions for 6 days, and three 24 hour urine specimens must be analyzed. Bauer and Aub's method is such a quantitative test.

Both types of tests for calcinuria have not been much used in children, since the disorders they may reveal occur only rarely during childhood. However, further study is needed to determine whether the significance of urinary calcium excretion in children is restricted to the same conditions as in adults, and whether these conditions are actually so rare in the early years of life. For this reason, the Sulkowitch and Bauer-Aub tests are described here in brief detail. To verify the abnormal calcium balance in resistant rickets, low phosphate rickets, and renal hyperparathyroidism, data on both urinary and fecal calcium are essential.

SULKOWITCH TEST (35)

Reagent. Oxalate buffer reagent. Into a 150 cc. volumetric flask transfer 2.5 Gm. oxalic acid, 2.5 Gm. ammonium oxalate, 5 cc. glacial acetic acid, and distilled water to make up to volume.

If the urine does not give an acid reaction to litmus paper, it must be made so by addition of a 50 per cent acetic acid solution.

Into a test tube are measured 5 cc. acid urine and 5 cc. oxalate buffer reagent, the tube is inverted and shaken, and the mixture is observed for turbidity (precipitation) after 2 minutes.

INTERPRETATION

Low turbidity or a minimal precipitate is considered a grade 1 response and is taken to indicate a normal calcium concentration. More pronounced turbidity or precipitation is graded 2, 3, or 4, with grade 4 being applied to a dense cloud of precipitated calcium.

According to Barney and Sulkowitch (35) a normal result is most helpful in ruling out hyperparathyroidism; an abnormal response, i.e., grades 2 to 4, supports the diagnosis of hyperparathyroidism and "should encourage one to make further studies; it should not, however, be used as a final court of appeal."

Recumbent adults with compound fractures excrete steadily increasing amounts of calcium in the urine for approximately 30 days, after which calcium excretion levels off and the level is maintained presumably until the patient becomes mobile (36). During the recumbent period the Sulkowitch test frequently gives positive results, signalizing the imminent danger of calcium phosphate and calcium oxalate stone formation. In the presence of renal dysfunction, the test loses its significance. Little is known about the effect of recumbency on the urinary calcium excretion of children.

CALCIUM EXCRETION TEST OF BAUER AND AUB (37)

The patient should be on a low calcium diet for at least 3 days before the test and for the 3 day test period, the daily intake of calcium and phosphate being maintained at about 0.11 Gm. and 0.4 Gm., respectively. Bauer and Aub have supplemented the data available in food tables (38) by analyzing the calcium content of a large number of foods (Table 36). An example of a low calcium diet, such as used by the authors, is given in Table 37.

TABLE 36
Calcium Content of Foodstuffs

Foodstuff	Ca content, Gm./100 Gm.	Foodstuff	Ca content, Gm./100 Gm.
Apple	0.010	Honey	0.004
Bacon, broiled	0.030	Klim	0.996
Banana	0.007	Liver	0.006
Bread, milk-free	0.011	Macaroni	0.018
Bread, regular	0.041	Orange juice	0.029
Bread, graham	0.060	Peaches	0.007
Chicken, white meat	0.016	Potatoes, raw	0.011
Chicken, dark meat	0.020	Potatoes, steamed	0.011
Chocolate	0.067	Rice, uncooked	0.007
Corn, canned	0.005	Rice, cooked	0.006
Crackers, graham	0.025	Shredded wheat	0.038
Crackers, Uneeda	0.022	Steak, fat	0.008
Cranberries	0.013	Steak, lean	0.008
Cream, 40%	0.069	Sugar	0.000
Farina	0.013	Tomatoes, fresh	0.007
Fudge, low calcium	0.003	Tomatoes, canned	0.005
Ginger ale, Cliquot	0.0005	Turkey	0.008
Goose	0.012	Vegex	0.019
Ham	0.022	Washed butter	0.000

From Bauer and Aub (37).

After the patient has been on the diet for at least 72 hours, 3 successive 24 hour specimens of urine are collected and preserved with chloroform or powdered thymol. The 3 specimens are measured separately, then thoroughly mixed in a large carboy, and just enough hydrochloric acid is added to the urine to render it definitely acid. An aliquot of the mixture is kept in the refrigerator for chemical analysis.

TABLE 37

Low Calcium Diet for an Adult of 60 Kilogram Body Weight. Total Calories 1,100; Calcium Content, 0.1 Gm.; Phosphorus Content, 0.582 Gm.

Meal	Food	Quantity, Gm.	Meal	Food	Quantity, Gm.
Breakfast.	Milk-free bread	30	2:30 P.M.	Ginger ale	400
	Butter fat	5	Supper...	Chicken, white meat	45
	Apple	100		Rice	75
	Orange juice	55		Corn	30
	Coffee	200		Milk-free bread	30
Dinner...	Steak	100		Butter fat	10
	Potatoes	100		Apple	100
	Tomatoes, fresh	150		Tea	200
	Milk-free bread	30	8 P.M....	Ginger ale	400
	Butter fat	10			
	Banana	100			
	Tea	200		NaCl per day	2

From Bauer and Aub (37).

The calcium determination may be carried out with 100 to 200 cc. of urine according to the (*a*) volumetric method of Shohl and Pedley (39), or (*b*) gravimetric method of McCrudden (40); or with 2 cc. of urine according to the titrimetric method of (*c*) Tisdall and Kramer (41), or (*d*) Sobel and Sobel (42).

Sobel and Sobel's method offers the definite advantage of direct acidimetric titration, without any previous ashing. This method is therefore described in detail below.

INTERPRETATION

According to Albright *et al.* (55), normal excretion in response to the diet amounts to about 65 mg. calcium per day. The same authors regard values between 125 and 200 mg. per day as highly suspicious, and an excretion in excess of 200 mg. per day as definitely abnormal.

Excessive calcinuria, as revealed by this test method, has essentially the same significance as the results of the Sulkowitch test, except that the results are more accurate.

A greatly increased urinary excretion of calcium which cannot be explained on other grounds, such as recumbency, supports the diagnosis of hyperparathyroidism. Increased urinary excretion of calcium is of particular interest in patients with slightly increased serum calcium levels, as it points to a potential hyperfunction of the parathyroids.

SOBEL AND SOBEL'S DETERMINATION OF CALCIUM IN URINE (42)

Reagents.

(*1*) Saturated solution of ammonium oxalate. Prepare at 40–50 C. and allow to cool to room temperature. Use the clear, supernatant fluid.

(*2*) 0.5 per cent ammonium oxalate solution.

(*3*) Patterson's indicator. Mix 100 cc. of 0.02 per cent methyl red and 30 cc. of 0.1 per cent methylene blue, and dilute to 500 cc.

(*4*) 0.04 per cent thymol blue solution.

(*5*) 10 per cent boric acid solution. Dissolve 10 Gm. boric acid in 100 cc. distilled water by heating. Since the solution is supersaturated at room temperature, reheat just before using to dissolve the precipitated boric acid, and use the hot solution.

The urine specimen to be used should be free of sediment. To clear up the sediment which forms in stored urine, boil a representative sample plus sediment, or the entire specimen if the sediment is coarse grained, with concentrated nitric acid in the proportion of 10 cc. of acid to 50 cc. of urine. After boiling to half the original volume, dilute the digest to known volume and analyze the sample.

Into a 15 cc. pyrex ungraduated centrifuge tube are pipetted 2 cc. of sediment-free urine and 1 cc. saturated ammonium oxalate. A drop of the thymol blue indicator is added, and the *p*H is adjusted to 3.0–3.3 with 6 *N* hydrochloric acid and ammonium hydroxide. The solution is allowed to stand for 3 hours, then is centrifuged at about 2,000 r.p.m. for 10 minutes. The supernatant fluid is decanted or carefully aspirated with a capillary pipet without disturbing the precipitate. The precipitate is then suspended in 3 cc.

of the 0.5 per cent ammonium exalate solution, centrifuged for 5–10 minutes, and the supernatant fluid carefully aspirated or decanted. The washed precipitate is dried in an oven at 100–110 C. and heated in a muffle furnace or sand bath at 475–525 C. for 30 minutes. The tube is then placed in a boiling water bath and 0.5 cc. of the hot boric acid solution is added; the precipitate dissolves completely in 1 to 2 minutes. The solution is diluted to 3 cc. with distilled water, 1–2 drops of Patterson's indicator are added, and the solution is titrated with 0.01 *N* sulfuric or hydrochloric acid. The end point is reached when the color is equal to that of a control tube, containing 0.5 cc. of the boric acid solution diluted to approximately 4 cc.

Calculation. The titration value represents the titrimetric equivalent of calcium: 1 cc. of 0.01 *N* acid used is equivalent to 0.2 mg. calcium.

$$\text{mg. Ca/100 cc. urine} = \frac{\text{cc. } 0.01\ N \text{ acid used} \times 20}{\text{cc. urine analyzed}}$$

Inorganic Phosphorus in Serum

The inorganic fraction (orthophosphate) forms the largest part of the acid-soluble portion of the phosphorus in serum (Fig. 29). For diagnostic purposes this fraction is the most important of the various phosphorus fractions in the blood. Parathyroid control keeps the plasma level of inorganic phosphate constant within narrow limits, and under normal conditions mobilization from the tissues and urinary excretion are balanced. The concentration of inorganic phosphate in serum is closely related to that of calcium (page 192). While it seems certain that, other factors remaining constant, the concentrations of calcium and phosphate ions in the serum bear a reciprocal relation to one another (43a), little is known as to the actual state of inorganic phosphorus in the serum. Deviations from normal occur infrequently, but if present they indicate marked anomalies in the distribution of phosphorus.

Inorganic phosphates are the principal vehicle transporting phosphorus within the organism. In addition to synthesis of the various organic ester compounds, many vital intracellular reactions depend on the constant metabolic turnover of phosphate. Lipmann (44) describes the "phosphate cycle" as being composed of: (*1*) the introduction of inorganic phosphate into ester linkage, (*2*) the generation of energy-rich phosphate bonds by oxidation-reduction, (*3*) the taking over and distribution of these phosphate bonds by cell catalysts (e.g., adenylic acid), and (*4*) the utilization of energy-rich phosphate compounds and the enzymic regeneration of inorganic phosphate.

Only a few methods, but with many modifications, have been recommended for measuring the inorganic phosphate fraction. After deproteinization of the serum, molybdate is added to the protein-free serum filtrates and color is developed by the reduction of the phosphomolybdic acid. The intensity of the color is in direct proportion to the inorganic phosphorus content of the serum filtrate. Most of the methods have been adapted for photoelectrometric measurement.

PEDIATRIC CONSIDERATIONS

Physiologically, inorganic phosphorus in serum is higher in children and adolescents than in adults. Seasonal fluctuations are common; phosphorus levels rise in the summer and fall during the winter months. While determination of inorganic phosphorus in serum is one of the most frequently performed tests in children, there are relatively few childhood diseases associated with changes in this fraction. However, the very absence of abnormal change may be very significant, particularly when the calcium level is aberrant.

The following are some of the procedures most frequently used in testing children, arranged according to the amount of serum or plasma required for a single analysis:

(*1*) 2 cc. of serum are required for the method of Bell and Doisy, as modified by Benedict and Theiss (45). It uses hydroquinone with sulfite as the reducing and color-producing agent.

(*2*) 0.5 to 1 cc. of serum is required for (*a*) the method of Kuttner and Cohen, as modified by Youngburg and Youngburg (6), using stannous chloride as the reducing and color-producing agent; and (*b*) the method of Fiske and Subbarow (46) using aminonaphtholsulfonic acid as the reducing and color-producing agent.

(*3*) 0.1 to 0.2 cc. of serum is required for the method of Kuttner and Cohen (9,47b), using stannous chloride as the reducing and color-producing agent.

A detailed description follows of two of the methods, *2b* and *3*.

PROCEDURES

FISKE AND SUBBAROW'S METHOD (46)

Reagents.

(*1*) 10 *N* sulfuric acid. Add 450 cc. concentrated sulfuric acid to 1,300 cc. distilled water.

(*2*) Molybdic acid solutions. No. 1: dissolve 25 Gm. ammonium molybdate in 200 cc. distilled water, rinse into a one-liter volumetric flask containing 300 cc. 10 *N* sulfuric acid, and make up to volume with distilled water. No. 2: dissolve 25 Gm. ammonium molybdate in 200 cc. distilled water, rinse into a one-liter volumetric flask containing 500 cc. 10 *N* sulfuric acid, and make up to volume with distilled water.

(*3*) 15 per cent sodium bisulfite solution. It should be free from turbidity; when freshly prepared, the solution may not filter clear, in which case allow the solution to stand for 2 to 3 days before filtering.

(*4*) 20 per cent sodium sulfite solution. Dissolve 200 Gm. crystalline sulfite ($Na_2SO_3 \cdot 2H_2O$) in 380 cc. distilled water. Remove any suspended matter by filtration, and keep the solution in a stoppered bottle.

(*5*) 0.25 per cent aminonaphtholsulfonic acid. The "technical" reagent (Eastman Kodak) has to be recrystallized. Dissolve 150 Gm. sodium bisulfite in 1 liter of water heated to about 90 C. Add 15 Gm. crude sulfonic acid, and shake the solution until all but the amorphous impurity has dissolved. Filter the hot solution through a large paper (about 32 cm. in diameter); cool the filtrate thoroughly under the tap, and add 10 cc. concentrated hydrochloric acid. Filter the precipitate by suction, wash with about 300 cc. distilled water, and finally with alcohol until the washings are colorless. Dry the purified sulfonic acid in air with the least possible exposure to light, then powder and transfer to a brown bottle.

Dissolve 5 Gm. of the dry powder in a brown, glass-stoppered, 300 cc. bottle with 195 cc. of 15 per cent sodium bisulfite and add 5 cc. of 20 per cent sodium sulfite. Stopper and shake the bottle until the powder has dissolved. More than 5 cc. of sulfite will be needed if the bisulfite solution is old; add 1 cc. at a time until solution is complete, taking care to add no more than is needed to dissolve the reducing agent. This solution should keep for about 2 weeks, if it is not exposed to the air.

(*6*) 7 per cent trichloroacetic acid solution.

(*7*) 10 per cent trichloroacetic acid solution.

(*8*) Standard phosphate stock solution. Dissolve 0.3509 Gm. monopotassium phosphate in water. Transfer the solution quantitatively to a one-liter volumetric flask, add 10 cc. of 10 *N* sulfuric acid and distilled water to the mark, and mix the contents by inverting the bottle. The standard keeps indefinitely. 5 cc. of the solution contains 0.4 mg. phosphorus.

Determination by Visual Colorimetry. Into a test tube are transferred 0.5 cc. serum or plasma and 4 cc. of 7 per cent trichloroacetic acid. The mixture is allowed to stand in the refrigerator for 15 minutes, and is then filtered. 2 cc. of the clear filtrate are transferred into a 10 cc. volumetric flask or tube, and 5 cc. of the molybdic acid solution No. 1, 1 cc. of sulfonic acid reagent, and water to the 10 cc. mark are added. This final solution, equivalent to 0.22 cc. of serum, is heated in a water bath at 37 C. for 5 minutes, cooled under the tap, and read in the colorimeter against the standard.

The standard phosphate solution is prepared by transferring 2 cc. of the phosphate stock solution, equivalent to 0.16 mg. phosphorus, into a 10 cc. volumetric flask. When the unknown sample is being made ready for colorimetry, 5 cc. molybdic acid solution No. 2 and 1 cc. of sulfonic acid reagent are added to this diluted phosphate standard. Heating, cooling, and transferring into the cup of the colorimeter are carried out as described for the sample solution. If required, more or less concentrated phosphate standards are prepared correspondingly.

Calculation.

$$\text{Mg. P per 100 cc. serum} = \frac{\text{standard}}{\text{unknown}} \times \frac{\text{mg. P in standard}}{\begin{array}{c}\text{cc. serum in final solu-}\\ \text{tion used in colorimetry}\end{array}} \times 100$$

The result of a blank determination, carried out similarly, is subtracted from the value obtained for the sample. The blank consists of 2 cc. of 7 per cent trichloroacetic acid, 5 cc. of molybdic acid solution no. 1, 1 cc. of sulfonic acid reagent, and water to make 10 cc.

Determination with the Evelyn Electrocolorimeter. In a test tube are mixed 0.5 cc. of serum and 9.5 cc. of 10 per cent trichloroacetic acid. The mixture is allowed to stand in the refrigerator for 15 minutes, and is then filtered through a Whatman filter no. 32. To 5 cc. of the filtrate are added 1 cc. molybdic acid solution no. 1, 0.4 cc. sulfonic acid reagent, and water up to 10 cc. The blank consists of 5 cc. of 10 per cent trichloroacetic acid, 1 cc. molybdic acid solution no. 1, 0.4 cc. sulfonic acid reagent, and water up to 10 cc.

After 15 minutes, the galvanometer is adjusted to 100 with the blank. A reading is taken, using filter no. 660; the reading of the sample is corrected, as shown on the card supplied with the apparatus, the corrected reading representing the value G.

Calculation. The concentration of inorganic phosphate in the serum, expressed in per cent, is determined directly from the value G on a previously prepared calibration curve for phosphate (47a).

Such a curve is obtained by determining the galvanometer reading for dilute solutions made from the standard phosphate stock solution. In a volume of 5 cc. the solutions contain from 0.0025 to 0.03 mg. of phosphate, corresponding to phosphate concentrations of 1 to 12 mg. per 100 cc. of serum. To the various sample tubes, each containing 5 cc. of a different phosphate dilution, are added 1 cc. molybdic acid solution no. 2, 0.4 cc. of aminonaphtholsulfonic acid, and water to make 10 cc. The blank consists of 8.6 cc. water, 1 cc. molybdic acid solution no. 2, and 0.4 cc. of aminonaphtholsulfonic acid. The calibration curve is best prepared on semilogarithmic paper with galvanometer readings of the various dilutions plotted on the logarithmic scale.

One can also calculate the analytic results by the formula

$$\text{mg. P per 100 cc. serum} = 100(L/K_2)$$

where L is $2 - \log G$, the value of L corresponding to any value of G being read from a table supplied with the galvanometer, and K_2 is the calibration constant as determined from the results of the above calibration procedure. Under the standarized conditions of the procedure outlined with the Evelyn photoelectrocolorimeter, K_2 equals 6.16 ± 0.15. Further details on calculations and calibration may be found in the instructions supplied with the apparatus.

KUTTNER AND COHEN'S METHOD (9,47b)

Apparatus.

(*1*) Electrocolorimeter.
(*2*) Test tubes, 12 × 120 mm., graduated at 1 and 2 cc.

Reagents.

(*1*) 7 per cent trichloroacetic acid.
(*2*) Molybdic sulfuric acid mixture.
(*3*) Stannous chloride solution; reagents (*1*) to (*3*) are the same as used by the authors for calcium determination (page 186).
(*4*) Standard phosphate solution (page 205).

Into a test tube are transferred 0.2 cc. serum or plasma and 7 per cent trichloroacetic acid to the 2 cc. mark. The tube is stoppered, shaken, and centrifuged for a few minutes. Then 0.5 cc. of the clear, colorless supernatant fluid is transferred into another tube, 0.4 cc. of the molybdic–sulfuric acid mixture is added with a micropipet, the contents are mixed by tapping the lower end of the tube sharply, 0.1 cc. diluted stannous chloride solution and water to the 2 cc. mark are added, and the tube is stoppered and immediately inverted. After 1 minute, the colored solution is transferred into the microcell of the electrocolorimeter, the zero reading having been previously determined. A blank sample is treated similarly.

Using the galvanometer reading of the sample, corrected for the reading of the blank, the concentration of inorganic phosphate in the analyzed serum, expressed in per cent, is computed on a calibration curve for phosphorus, prepared as described on page 206.

INTERPRETATION

The normal range and average levels of the inorganic phosphate in serum from birth to the age of 40 are given in Figure 28. The concentration is higher in the newborn than at any other age, averaging 6.03 mg. per hundred cubic centimeters, with a range of 3.5 to 8.6 mg. (48), and throughout childhood there is a definite physiologic hyperphosphatemia, as compared to adult levels.

Concentrations more than 1 mg. per hundred cubic centimeters above or below normal average values should be considered abnormal, except in infants below the age of 10 months, in whom the normal range is wider than in older children. In infants, therefore, only values over 1.5 mg. below or above the average figures are indicative of deviations from the normal.

Hypophosphatemia is pathognomonic for uncomplicated rickets. In rickets associated with other metabolic disorders, the reduction may be less definite, or even reversed to hyperphosphatemia. Table 35 gives some of the typical findings. Lowered inorganic phosphate levels are also usually found in hyperparathyroidism, the degree of hypophosphatemia varying frequently, in reciprocal relation to the degree of hypocalcemia.

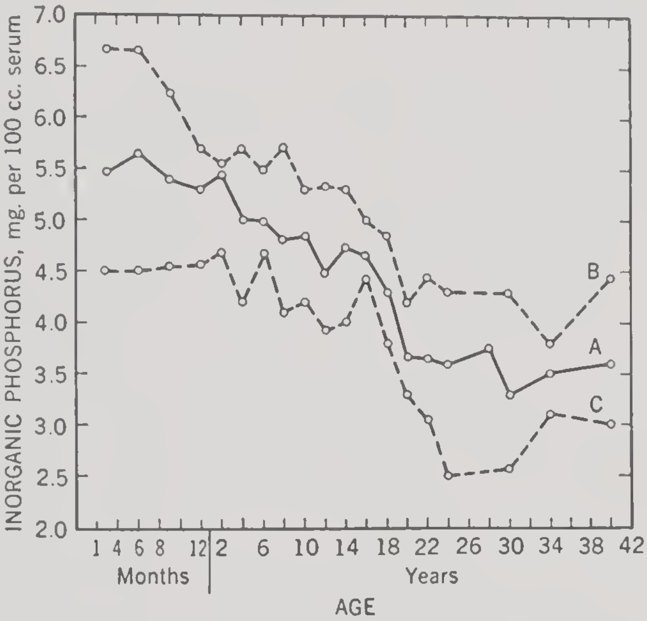

Fig. 28. Physiologic variations with age in the level of inorganic phosphorus in serum. A: Mean values. B–C: Limits of normal range. According to Bullock (48a).

Hyperphosphatemia is almost always present in infantile (rachitic) tetany, and in tetany due to hypoparathyroidism (Table 35B). In intestinal obstruction, particularly in pyloric stenosis (pylorospasm), the rise in the inorganic serum phosphate is characteristic; it rises and falls in reciprocal relation to the serum chloride concentration. An increase in inorganic serum phosphate is also found in the later stages of chronic nephritis.

Phosphorus Fractions in Blood

Unlike calcium, which is present almost exclusively in the plasma, phosphorus is present both in the red corpuscles (intracellular) and in the plasma (extracellular). Chemically, the phosphorus in the blood can be partitioned into the acid-soluble fractions, which include inorganic phosphorus and organic phosphorus esters, and the ether–alcohol soluble fractions, consisting of lipoid phosphorus and nucleic acid phosphorus. The acid-soluble phosphorus fractions are so distributed that the erythrocytes carry all the organic and some of the inorganic phosphorus, while the plasma carries only the inorganic form. The ether-soluble fraction is a constituent of both plasma and erythrocytes. Figures 29 and 30 illustrate the phosphorus partition in plasma and erythrocytes and the resulting concentrations of the various fractions in whole blood.

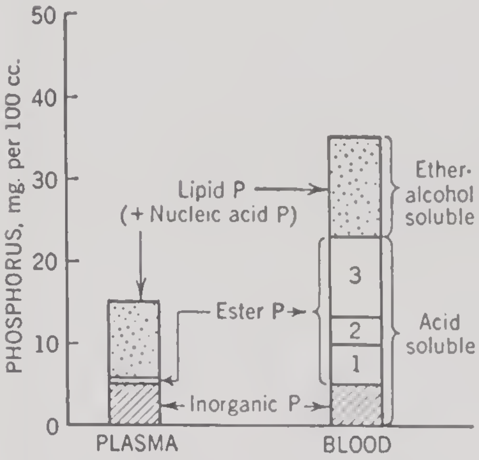

Fig. 29. Partition of phosphorus in plasma and in whole blood in a normal 2 month old child (49).

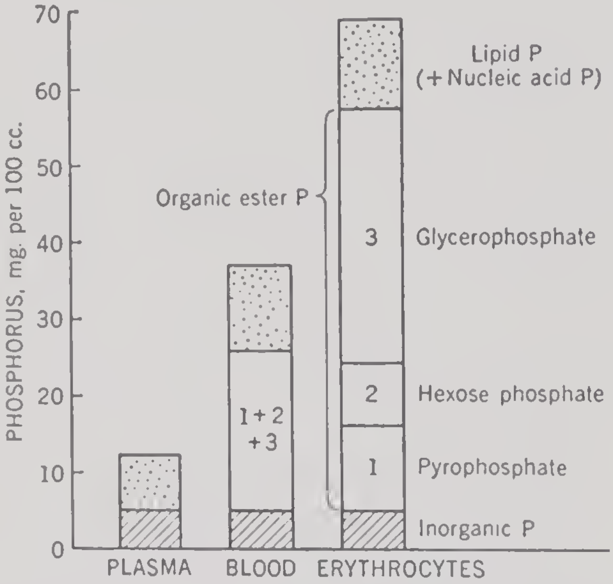

Fig. 30. Phosphorus fractions in the plasma, whole blood, and erythrocytes of a normal 1 year old child (49).

(1) phosphorus from adenosine triphosphate (pyrophosphate); (2) phosphorus from hexose esters (hexose monophosphate plus hexose diphosphate); (3) phosphorus from glycerophosphate.

The concentrations of the organic phosphorus fractions vary greatly in the same individual, and in different individuals of the same age, so that small or moderate deviations from the normal are not very significant. The level of organic esters, particularly, is inconstant, the concentration varying with changes in the rate of synthesis and decomposition. The enzymic action of phosphatase splits the ester compounds by hydrolysis into the inorganic compound (phosphate) and organic compounds (alcohol, hexose); this process is termed "phosphatolysis." Synthesis is accomplished either by the same enzyme, or by the enzyme phosphatase. The esters take part in numerous vital metabolic processes, such as oxidation-reduction, sugar metabolism (glycolysis), muscular contraction, and acid-base regulation. To some extent, they also serve as carriers of phosphorus, which may be utilized in tissues or may leave the body in urine and feces (50).

This brief description will serve to suggest the scope of the physiologic functions of the ester compounds and the obvious clinical consequences of disturbed function. Changes in the concentration of the individual esters and of their sum total may occur in so many metabolic disorders that their diagnostic significance is rather limited, and the fractional analysis of phosphorus compounds in the blood is of theoretic rather than of practical interest.

For methods of analysis and their results, as obtained in rickets, tetany, pyloric stenosis, diabetes, and chronic nephritis, the reader is referred to the work of Guest and co-workers (50). A relatively simple method (49,51) of estimating the partition of phosphorus in the blood comprises three procedures: (*1*) Serum is analyzed for total and inorganic phosphorus, the difference between the two figures thus obtained representing lipoid phosphorus. (*2*) Whole blood is analyzed for total, acid-soluble, and inorganic phosphorus; organic ester phosphorus is calculated as the difference between the values for acid-soluble and inorganic phosphorus, the lipoid phosphorus as the difference between values for total and acid-soluble phosphorus. (*3*) For phosphorus partition in erythrocytes, each fraction is calculated from the respective figures obtained for serum and whole blood, using the relative volume of packed blood cells as the basis of calculation. The formula is:

$$\text{Solute concentration in red cells} = \frac{S_B - (S_P V_P)}{V_c}$$

where S_B and S_P represent the concentration of the phosphorus fraction in whole blood and plasma, respectively, and V_c and V_P the volume (as $x/100$) of red cells and plasma, respectively.

Phosphate Tolerance Test

Warkany's phosphate tolerance test (52) is one of the few existing function tests of mineral metabolism. As Warkany has shown, the normal response of a child to an oral test dose of orthophosphates is an increase in the inorganic serum phosphate.

After a 10 hour fast the child is given by mouth 0.5 Gm. sodium diphosphate per kilogram of body weight, dissolved in water. Blood samples are obtained before and 1, 2, and 3 hours after ingestion of the test dose. The inorganic serum phosphate is measured by one of the micromethods (page 203).

By means of this test, the shape of the normal phosphatemic curve has been established, and abnormal reactions have been studied. Normally, the test dose brings a rise of 2.5 to 4.5 mg. per hundred cubic centimeters, the average rise being 3.4 mg. (Fig. 31).

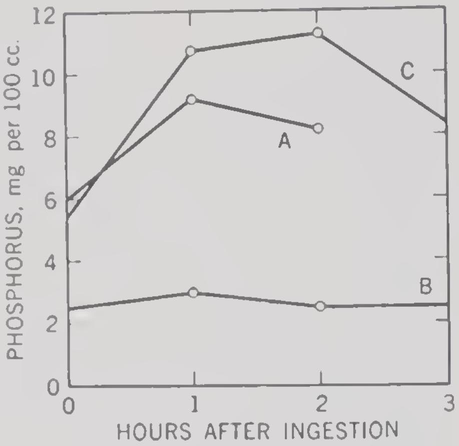

Fig. 31. Phosphatemic curves in serum after a standard loading dose, administered orally. A: Normal 18 month old child. B: 14 month old child with active rickets. C: Same child, 20 days later, after vitamin D therapy. From Warkany (52).

In rickets, there is an abnormally low rise in response to the test dose, the average increase being 0.4 mg. per hundred cubic centimeters (Fig. 31), sometimes no increase at all occurs. Oral ingestion of vitamin D for a week or longer restores the phosphatemic response to normal or above normal. Warkany holds that the shape of the curve indicates the vitamin D level in the tissues.

Phosphatase Activity in Serum

Phosphatases are enzymes of the esterase group which act as catalytic agents in the hydrolysis or synthesis of phosphoric esters. It is not yet definitely known whether a single enzyme acts on the various ester compounds of phosphoric acid found in the human blood, or whether there is a specific phosphatase for each ester substrate. Recent investigations, however, have resulted in a differentiation between alkaline and acid phosphatases, with an optimum activity at about *p*H 9 and *p*H 5, respectively.

Alkaline phosphatases are widely distributed in body organs and fluids, with particularly high concentration in the intestinal mucosa, kidneys, and bones (ossifying cartilage). According to Kay (53), there is fairly convincing evidence that the alkaline phosphatases found in these organs and those present in blood plasma and leukocytes are identical.

TABLE 38
Substrate, Optimal Acidity, and Unit of Measurement Employed in the Various Methods of Estimating Serum Phosphatase

Method, according to	Ester substrate	Optimal acidity, *p*H	1 unit of phosphatase activity is equivalent to enzymic liberation of
Kay (58a)	β-Glycerophosphate	7.6	1 mg. P at 38 C. in 48 hrs.
Lundsteen and Vermehren (58b)	β-Glycerophosphate	8.87	1 mg. P at 37 C in 24 hrs.
Jenner and Kay (58c)	β-Glycerophosphate	8.8	1 mg. P at 38 C. in 3 hrs.
Roberts (58d)	β-Glycerophosphate	8.9	1 mg. P at 38 C. in 2 hrs.
Bodansky (59)	β-Glycerophosphate	8.6	1 mg. P at 37 C. in 1 hr.
	β-Glycerophosphate	5.0	1 mg. P at 37 C. in 1 hr.
King and Armstrong (60)	Phenylphosphate	9.0	1 mg. phenol at 37.5 C. in ½ hr.
Gutman and Gutman (62)	Phenylphosphate	4.9	1 mg. phenol at 37 C. in 1 hr.
Huggins and Talalay (62a)	Phenolphthalein-phosphate	9.7	0.1 mg. phenolphthalein at 37 C. in 1 hr.
	Phenolphthalein-phosphate	5.4	0.1 mg. phenolphthalein at 37 C. in 1 hr.
Bessey, Lowry, and Brock (62b)	*p*-Nitro phenylphosphate	10.3	See page 222

Acid phosphatases are present mainly in the spleen, brain, erythrocytes, and prostate gland, where their concentration is particularly high.

Among the physiologic functions in which the phosphatases participate are the intestinal absorption of sugar and fat, the reabsorption of sugar in the renal tubules, the renal excretion of phosphates, the calcification of bone and teeth, and the intermediate processes of sugar metabolism. Phosphatases also take part in the vital intracellular reactions which depend on phosphorylation and dephosphorylation.

The presence of both alkaline and acid phosphatase in human plasma has been demonstrated. Under normal conditions, the phosphatase level varies within narrow limits. A rise or drop above or below the normal phosphatase levels in serum indicates changes in their genesis. Based on Robison's theory (54) that bone is the main source of alkaline phosphatase, variations in alkaline phosphatase in serum are generally considered to be a result of changes in the ossifying activity in bone (55). However, since it is believed (56,57) that the enzyme also originates in the liver and is excreted in the bile, increased alkaline phosphatase in serum may also indicate the presence of hepatic dysfunction and biliary obstruction. So far, the only known source for an abnormally increased acid phosphatase in serum is metastasizing cancerous tissue of the prostate gland (58c).

The activity of phosphatase in serum is commonly measured by determining the amount of inorganic phosphorus or phenol liberated from their respective ester compounds by a known amount of added serum at the *p*H of optimal activity and in a unit of time. The difference between the values of the split product, as determined before and after the enzymic hydrolysis, represents the degree of phosphatase activity. Unfortunately, the units of phosphatase activity, as adopted by different workers and measured by different methods, are not uniform, so that only results obtained by identical methods can be readily compared. Table 38 lists the various units of phosphatase activity and their definition. Information on serum alkaline phosphatase activity in children has been obtained chiefly by means of Bodansky's method (59) and that of King and Armstrong (60), both requiring 1 cc. of serum for each determination. Yet, for pediatric use, the new micromethod of Bessey, Lowry, and Brock (62b) seems to be far preferable; it is a simple and rapid determination and requires only 5 c.mm. of serum. Since its frequent use in children is anticipated, the method is outlined at the end of this section.

Determination of acid phosphatase in serum (62), which as yet has not attained clinical importance in diagnosing childhood diseases, will not be further discussed.

BODANSKY'S METHOD OF ALKALINE SERUM PHOSPHATASE DETERMINATION (59)

Apparatus. Colorimeter, or electrocolorimeter.

Reagents.

(*1*) Buffered glycerophosphate solution. Transfer into a 100 cc. volumetric flask in succession about 3 cc. petroleum ether (b.p. 20–40 C.), about 80 cc. distilled water, 0.5 Gm. β-glycerophosphate, 0.424 Gm. sodium diethylbarbiturate, and distilled water to volume (read at interface between substrate and petroleum ether); empty contents into a 100 cc. glass-stoppered pyrex bottle containing about an inch of petroleum ether.

(*2*) 10 per cent trichloroacetic acid.

(*3*) 7.5 per cent sodium molybdate solution.

(*4*) 10 *N* sulfuric acid. Dilute 290 cc. concentrated sulfuric acid (sp. gr. about 1.83) to 1 liter; standardize the solution against 10 *N* sodium hydroxide and dilute if necessary; store in refrigerator.

(*5*) 60 per cent stannous chloride solution. To 15 Gm. stannous chloride add concentrated hydrochloric acid (approximately 36 per cent) to a volume of 25 cc. Store in the refrigerator.

(*6*) Diluted stannous chloride solution. Dilute 1 cc. of the 60 per cent solution to 400 cc. with distilled water; keep in refrigerator when not in use.

(*7*) Standard phosphate stock solution. Transfer into a 250 cc. volumetric flask in succession 110 mg. potassium diphosphate, 1 cc. concentrated sulfuric acid, and distilled water to volume; 1 cc. contains 0.1 mg. phosphorus.

(*8*) Dilute standard phosphate solution. Prepare fresh before use. Dilute 10 cc. stock solution to 300 cc. and add 1 drop toluol; 6 cc. contain 0.02 mg. phosphorus.

(*9*) Molybdic–sulfuric acid mixture. To an equal volume of the cold 10 *N* sulfuric acid, add, while mixing, sodium molybdate solution; if the solution is properly prepared, it is free of even the slightest tinge of yellow.

About 4 cc. of whole blood are collected into a centrifuge tube, allowed to clot at room temperature, and centrifuged. The serum is removed by aspiration, centrifuged, and the supernatant fluid is used for analysis. If serum is kept in the refrigerator for 24 to 48 hours, its phosphatase activity is found to be about 10 to 15 per cent higher than when the serum is analyzed fresh.

Preparation of "Serum Inorganic P" Filtrate. 4.5 cc. of the trichloroacetic acid are added to 0.5 cc. of serum in a test tube, the contents are well mixed, and after a few minutes are filtered through a 9 cm. filter paper (Whatman no. 42, or other ashless grade).

Preparation of "Total Inorganic P" Filtrate. This is the sum of serum inorganic phosphorus and the inorganic phosphorus liberated from the buffer substrate by the serum phosphatase. A test tube, preferably glass-stoppered, containing 5 cc. of the glycerophosphate solution is placed in a water bath at 37 C. for a few minutes, and 0.5 cc. of serum are then added, the tip of the pipet being kept about 1 cc. above the surface of the liquid. The tube is inverted once or tapped, to mix the contents, and replaced in the water bath. Exactly 1 hour later, it is removed from the bath, cooled in ice water for about 2 minutes, and 4.5 cc. of trichloroacetic acid are added. The contents are mixed, allowed to stand for a few minutes, and then filtered, as above.

Phosphorus determination is carried out in aliquots of the two filtrates by any convenient method. When the Kuttner-Lichtenstein method is followed, as Bodansky recommends, 1, 2, or 3 cc. of each filtrate are analyzed, according to the phosphatase concentrations expected. Each of the 2 aliquots chosen is transferred into a properly labeled tube and made up with water to a total volume of 6 cc. Two blanks are also prepared, one containing 6 cc. of water, the other 6 cc. of trichloroacetic acid. Finally, 6 cc. of standard phosphate dilution are measured into a test tube. To each of the 5 tubes, 2 cc. of molybdate solution and 2 cc. of diluted stannous chloride solution are added, the contents of each tube being mixed by tapping during the addition. Colorimetric or electrocolorimetric readings may be taken immediately after addition of the reagents, or at any time within 2 hours (see page 207).

Visual Colorimetry. When 6 cc. of diluted phosphate solution are used for comparison, the standard is equivalent to 0.02 mg. phosphorus. Under the given quantitative conditions, calculation for the phosphate content of the filtrates is as follows:

Serum inorganic P:

$$\frac{\text{Standard reading}}{\text{Unknown reading}} \times 0.02 \times \frac{5}{\text{cc. filtrate used}} \times \frac{100}{0.5} = \text{mg. P per 100 cc.}$$

Total inorganic P:

$$\frac{\text{Standard reading}}{\text{Unknown reading}} \times 0.02 \times \frac{10}{\text{cc. filtrate used}} \times \frac{100}{0.5} = \text{mg. P per 100 cc.}$$

Phosphatase:

$$\underset{\text{per 100 cc.}}{\text{Total inorganic P}} - \underset{\text{per 100 cc.}}{\text{serum inorganic P}} = \underset{\text{per 100 cc. of serum}}{\text{phosphatase units}}$$

For routine clinical purposes, corrections need not be made for deviations from Beer's law and for the effects of the reagents. If required, these corrections can be obtained from tables in the original description of the method (59).

Electrocolorimetry. A calibration curve for inorganic phosphate is prepared, as described on page 206. The concentration of phosphate, in milligrams per hundred cubic centimeters, is read directly from the prepared curve. Here again, the difference between total and serum inorganic phosphorus per hundred cubic centimeters represents phosphatase units in 100 cc. of serum. The definition of 1 unit of phosphatase activity, as adopted for this method, is given in Table 38.

KING AND ARMSTRONG'S DETERMINATION OF ALKALINE SERUM PHOSPHATASE (60)

Reagents.

(*1*) Buffered phenylphosphate substrate of *p*H 9.0. Dissolve 1.09 Gm. disodium monophenylphosphate and 10.3 Gm. sodium diethyl barbiturate (veronal) in water, and dilute to 1 liter. Add 2 to 3 cc. of chloroform, as a preservative, transfer the solution into a glass-stoppered bottle, and keep in the refrigerator.

(*2*) Phenol reagent of Folin and Ciocalteu (61). Place 50 Gm. sodium tungstate, 12.5 Gm. sodium molybdate, and 350 cc. water in a 1-liter Florence flask, and add 25 cc. of 85 per cent phosphoric acid and 50 cc. concentrated hydrochloric acid. Connect the flask with a reflux condenser by means of a cork or rubber stopper wrapped in tin foil, and boil the contents of the flask gently for 10 hours. At the end of this period add 75 Gm. lithium sulfate, 25 cc. water, and a few drops of liquid bromine. Boil the mixture, without the condenser, for about 15 minutes to remove the excess bromine, cool under running water, and dilute to 500 cc. with distilled water. Filter the diluted solution into a well-stoppered amber bottle. The solution should be completely free of any greenish tinge; such a tinge, a sign that blue reduction products are present, lessens the accuracy of the colorimetric determination. Keep the reagent well protected against dust, as organic materials will gradually produce slight reduction.

(*3*) Dilute phenol reagent. Dilute 1 volume of the phenol reagent with 2 volumes of distilled water just before it is to be used.

(*4*) 20 per cent sodium carbonate solution. Dissolve 20 Gm. anhydrous sodium carbonate in water and dilute to 100 cc.

(*5*) Standard phenol stock solution. Dissolve 0.1 Gm. crystalline phenol in 0.1 *N* hydrochloric acid and make up to 100 cc. with the hydrochloric acid. The solution keeps indefinitely. It is standardized by titration with iodine as follows (43b):

> Transfer 10 cc. of the stock solution into a 100 cc. flask, add 20 cc. 0.1 *N* iodine solution, stopper the flask and let stand at room temperature for 30 minutes. Add 2 cc. concentrated hydrochloric acid and titrate the excess iodine with 0.1 *N* sodium thiosulfate solution. The cubic centimeters of iodine added minus the cubic centimeters of thiosulfate used in titration represent the amount of iodine kept by the phenol. 1 cc. of 0.1 *N* iodine corresponds to 1.567 mg. phenol.

(*6*) Dilute phenol standard solution. On the basis of the titration, dilute the standard phenol stock solution with distilled water so that 1 cc. contains 0.1 mg. phenol. Under refrigeration, the solution keeps for months.

Technic. Into each of 2 test tubes, marked A and B, are transferred 10 cc. of the phenylphosphate substrate, and the test tubes are placed in a water bath at 37.5 C. for about 5 minutes. Then 0.5 cc. of serum is added to tube A, the tube is stoppered, inverted once, incubated at 37.5 C. for exactly 50 minutes, 4.5 cc. of the phenol reagent are added, the contents are mixed, and finally filtered.

To test tube B are transferred 0.5 cc. of serum and 4.5 cc. of the phenol reagent and the contents are mixed and filtered. The deproteinized filtrates of samples A and B are now ready for the determination of phenol. Before proceeding with the determination, a standard is prepared by measuring into a 50 cc. volumetric flask, in sequence, 5 cc. of the dilute phenol solution, 15 cc. of the dilute phenol reagent, and water to the mark.

Into 3 test tubes, properly labeled, are transferred 10 cc. of filtrate from tube A, 10 cc. of filtrate from tube B, and 10 cc. (containing 0.1 mg. phenol) of the freshly prepared standard, respectively. 2.5 cc. of the sodium carbonate solution are added to each of the 3 tubes, the contents are mixed, and the tubes are placed in the water bath at 37.5 C. for 5 minutes to develop the color. The 2 sample solutions are then compared with the standard in a colorimeter. The phosphatase units in each of the 2 samples are calculated as follows:

$$\frac{\text{Standard}}{\text{Unknown}} \times 0.1 \times \frac{15}{10} \times \frac{100}{0.5} = \text{phosphatase units per 100 cc.}$$

If very high phosphatase activity is expected, the serum is diluted tenfold, and 0.5 cc. of the dilution is analyzed. To obtain the number of phosphatase units in 100 cc. of serum, the number of units found in unknown sample in tube B are subtracted from the number of units determined in unknown sample in tube A. For the definition of one unit of phosphatase activity, as adopted for this method, see Table 38.

INTERPRETATION

Table 39 gives the normal results, as determined by the two methods just described. In children, variations of the normal range in relation to age are noteworthy. The average normal level is lowest in the newborn, namely, 7.1 Bodansky units (63). It rises to a maximum of about 15 Bodansky units during the first month of life, and remains near the upper limit of the normal range until well into the second year (64). In children between the ages of 2 and 10, the level is usually below 10 Bodansky units, whereas between the ages of 10 and 15 the level rises again to 10 to 14 Bodansky units, or 20 to 25 King-Armstrong units. During the following years the phosphatase level drops to the normal range found in adults.

TABLE 39
Normal Serum Phosphatase Values

Age	Alkaline phosphatase		Acid phosphatase
	Bodansky units	King-Armstrong units	Gutman units
Infants	10–20	—	—
Children	5–14	15–20	0.5–2.5
Adults	2–3.5	5–10	0.5–2.5

According to Jaffe and Bodansky (65).

Values above 18 Bodansky units or 30 King-Armstrong units in children over 1 year of age may be considered abnormal, whereas in infants only values above 25 Bodansky units should be so considered. The corresponding figure for King-Armstrong units has not yet been ascertained, but it is probably around 35.

Any value below 4.5 Bodansky units or 10 King-Armstrong units is a sign of abnormally low activity. Though not necessarily pathologic, such low figures should receive special attention.

Abnormally increased phosphatase activity is found in two groups of diseases—those involving osteoblastic activity in the bones, and those affecting liver function. Of the first group, rickets is the most important in pediatric practice. Children with mild rickets have a phosphatase activity of 20 to 30 Bodansky units; in severe rickets this rises to 60 units or more. Expressed in King-Armstrong units, the values are approximately 35 to 50, and 70 units, respectively. It seems doubtful whether the level of phosphatase in serum is a better criterion of the activity of the disease than the inorganic phosphorus concentration in serum.

Serum phosphatase activity is also apt to be increased in other diseases in which bones are involved (58), such as osteomalacia (adult rickets); renal hyperparathyroidism (renal rickets); Recklinghausen's disease; Paget's disease; osteogenic sarcoma; and osteoplastic carcinomatous metastases. According to Jaffe and Bodansky (65), "in those tumorous disorders in which the skeletal lesions are purely lytic (for instance in multiple myeloma) the phosphatase value is usually not above normal."

When abnormally increased phosphatase activity occurs in liver disease, the presence of obstructive rather than of parenchymatous disorders is suggested. Phosphatase determination shows whether a bile constituent has leaked into the blood, but it is not a test of the liver's excretory capacity. Any liver injury or obstruction permitting bile to enter the circulation causes an elevation of the serum phosphatase activity (56). The possible presence of hepatic disease should always be considered when phosphatase activity in serum is abnormally increased. The test is being increasingly used for differential diagnosis of obstructive and hepatic jaundice. Its use with other liver function tests is described on page 27.

Unusually low serum phosphatase values are suggestive of hypothyroidism. In children they are of greater diagnostic significance (page 448) than other clinical tests for thyroid impairment (66).

BESSEY, LOWRY, AND BROCK'S DETERMINATION OF ALKALINE SERUM PHOSPHATASE (62b)

Apparatus.

Spectrophotometer or photoelectric colorimeter, adapted to 0.5 cc. volume measurements.

Constriction pipets (Long-Levy), 5 and 50 c.mm.
Capillary tubes, 7–10 cm. long, 1.5–2.0 mm. in outer diameter.
Serologic tubes, 6 × 50 mm.
Wire rack to hold the serologic tubes.

Reagents.

(*1*) Buffer solution. Dissolve 7.5 Gm. glycine and 95 mg. magnesium chloride in 700–800 cc. of distilled water, add 85 cc. *N* sodium hydroxide, and dilute to 1 liter.

(*2*) Substrate solution. Prepare 0.4 per cent disodium *p*-nitrophenyl phosphate in 0.001 *N* hydrochloric acid. (The commercially available product at present contains about 50 per cent inert material; hence, a double amount of this preparation should be used. If desired, the compound may be purified by recrystallization from hot 87 per cent alcohol.) If the *p*H of the solution is not between 6.5 and 8.0, adjust with acid and base. To test for free phenol, dilute 1 cc. with 10 cc. of 0.02 *N* sodium hydroxide and measure the light absorption at 415 mμ. If the extinction is greater than 0.08 (i.e., light transmission of less than 83 per cent for a 1 cm. light path or 70 per cent for a 2 cm. light path), remove free phenol by extracting reagent *2* two or three times with equal volumes of water-saturated butyl alcohol, and once with water-saturated ether, finally aerating off traces of ether. Store in the refrigerator. Re-extract when the solution fails to pass the above test.

(*3*) Complete reagent. Mix equal parts of reagents *1* and *2*. If necessary, adjust the *p*H to 10.3 or 10.4 with a little strong sodium hydroxide or hydrochloric acid. Store in the refrigerator, or better, store frozen. When 2 cc. of this reagent, after addition of 0.02 cc. *N* sodium hydroxide have an extinction (1 cm.) greater than 0.1, either discard or extract with butyl alcohol and ether as above, and readjust the *p*H.

(*4*) Standards. Prepare solutions containing 1, 2, 4, 6 millimoles per liter of *p*-nitrophenol, mol. wt. 139.1.

Collection of Serum Sample (62c). Blood is collected from the finger or ear lobe into a capillary tube described above, care being taken to wipe off the first droplet of blood and all traces of alcohol before taking the sample. Squeezing the finger is permissible. The tube is filled about three-quarters by capillarity. The tube is then tipped until the blood runs to the middle, taking care to keep one end dry. The ends are sealed as follows: A stick of picene is softened in a flame (match, alcohol lamp) and applied to the dry end of the capillary tube which has also been warmed in the flame. The other end of the capillary is capped with a little picene without warming the tube, since this might produce hemolysis. The capillary is centrifuged 5 to 10 minutes at 3,000 r.p.m., with the tightly sealed end down. When ready to make the determination, the top of the capillary above the serum is removed after scratching with a diamond point. A second scratch is made just above the red cells and the serum segment is removed. The serum can now be drawn into any appropriate pipet.

Procedure with 5 c.mm. of Serum. Using a constriction pipet, 5 c.mm. of serum are transferred from the capillary tube to the bottom of a small serologic tube in a wire rack. The rack is immersed in a shallow pan of ice water and 50 c.mm. of ice-cold reagent *3* are added to the tube with a constriction pipet. The contents of the tube are then mixed by tapping with the finger, care being taken not to warm the tube by so doing. The rack is now immersed in a water bath at 38 C. to cover the bottom half of the tubes. After 30 minutes the rack is again placed in the pan of ice water and 0.5 cc. of 0.02 N sodium hydroxide is added to the tube with sufficient force to mix the sample. The mixture is now transferred to a colorimeter tube and read at 400–420 mμ. This reading is designated R_1.

After this initial reading, 2–4 c.mm. of concentrated hydrochloric acid are added to the colorimeter tube with a graduated pipet (drawn out tip) and a second reading is made, designated R_2.

Standards and blanks are provided by treating 5 c.mm. volumes of standards and of distilled water exactly like the serum sample.

Procedure with 20 c.mm. of Serum. Volumes of serum, reagent *3*, and 0.02 N sodium hydroxide are increased to 20 c.mm., 200 c.mm., and 2 cc., respectively. Otherwise, the procedure is nearly identical with that described for 5 c.mm. of serum. The sample may be incubated directly in $^3/_8$ inch photocolorimeter tubes. 1 drop of 5 N hydrochloric acid is added before the second reading.

Procedure with 0.1 cc. of Serum. The volumes used are: 0.1 cc. serum, 1 cc. reagent *3*, and 20 cc. 0.02 N sodium hydroxide. A sufficiently large tube, containing 1 cc. of reagent is placed in the water bath and allowed to come to temperature before the serum is added. Exactly 30 minutes after addition of the serum, sodium hydroxide is added.

Calculation. The degree of splitting has been found to be proportional to the concentration of enzyme; a strictly linear relationship, however, between degree of splitting and time does not exist for more than about 30 minutes.

R_1 and R_2 are converted into optical densities (2 — log per cent transmission), designated D_1 and D_2, respectively.

$$D_1 - D_2 = D_c \text{ (corrected density)}$$

The serum values are calculated from a standard curve constructed from the corrected densities (D_c) of the various standard dilutions (page 206).

The result is expressed in "millimole units." One such unit is defined as the phosphatase activity which will liberate 1 millimole of nitrophenol per liter of serum per hour. Therefore, since the standard incubation time is only 30 minutes, the 1, 2, 4, and 6 millimole standards are equivalent to serums with activities of 2, 4, 8, and 12 millimole units.

From the authors' studies, it would appear that the ratio of Bodansky units to millimole units is 1.79, the ratio of King-Armstrong units to millimole units 7.3. For the time being, therefore, the interpretation of phosphatase values as given on page 218, may be utilized for evaluating results obtained by the present procedure.

SODIUM AND POTASSIUM

An abnormal potassium or sodium balance and a changed cation pattern in the plasma are manifestations of failure in the regulation of (*1*) acid-base equilibrium, (*2*) water balance, and (*3*) electrolyte balance. These mechanisms can be tested without resorting to an assay of sodium and potassium in urine and feces or in blood plasma. Thus, acidosis and alkalosis can be verified by measuring the plasma carbon dioxide content (page 240), and analysis of the electrolyte pattern in plasma, including sodium and potassium content, need only be done in special cases. Primary disturbances of water exchange also can be recognized by less cumbersome methods than study of sodium and potassium balances. The only feasible clinical method is a rough measurement of sodium balance by analysis of urinary sodium excretion (page 223). If the electrolyte balance itself is one of the mechanisms principally affected, as, for example, in adrenocortical deficiency, the serum sodium and potassium levels and urinary sodium excretion are diagnostically significant. However, the new function test for adrenocortical activity (page 460), the most sensitive test method of all does not require the determination of base in serum or urine. There are, nevertheless, some clinical situations in which the determination of sodium or potassium levels in serum, or of both, may be necessary.

Potassium in Urine and Serum

The method commonly used in clinical laboratories is that of Kramer and Tisdall (66a). Its description may be found in manuals of clinical chemistry, such as that of Levinson and MacFate (10); Kramer (66b), in a recent review of the subject of potassium determination, critically appraises modifications of the original method, and supplies answers to questions on choice of method, technic, and interpretation.

Sodium in Urine and Serum

The procedure to be described is that of Butler and Tuthill (66c); it applies the Barter-Kilthoff method to biologic material. Their claim that the method is less laborious, less expensive, and more accurate than other procedures has been widely confirmed.

DETERMINATION OF SODIUM IN URINE (66c)

Reagents.

(*1*) Uranium–zinc acetate reagent. Solution no. 1: to 80 Gm. sodium-free uranium acetate, $UO_2(C_2H_3O_2)_2 \cdot 2H_2O$, add 48 Gm. or 46 cc. of 30 per cent acetic acid (per cent by volume) and water to make 520 Gm. Solution 2: to 220 Gm. zinc acetate, $Zn\ (C_2H_3O_2) \cdot 2H_2O$, add 24 Gm. or 23 cc. of 30 per cent acetic acid and water to make 520 Gm.; cover both solutions and warm on a steam bath until, with stirring, solution is complete. Mix the two solutions while still hot, and let stand for 24 hours before using. If a yellow precipitate has not appeared, add 0.2 Gm. precipitated uranyl zinc sodium acetate, in order to saturate with this triple salt. Shake the mixture several times before using, and filter to assure saturation at the temperature of the analysis.

(*2*) 1 per cent alcoholic solution of phenolphthalein.

(*3*) Powdered corrosive mercuric chloride.

(*4*) Powdered calcium hydroxide.

(*5*) Saturated solution of ammonium perchlorate.

(*6*) 95 per cent alcohol saturated with precipitated uranyl sodium zinc acetate.

Procedure for Urine of Ordinary Sodium Content. Approximately 6 cc. of urine are measured into a small flask; 1 drop of phenolphthalein and 0.2 Gm. of calcium hydroxide are added. If the urine contains protein, 10 cc. of urine instead of 6 cc. are taken, and 0.05 Gm. of mercuric chloride is added before the addition of the phenolphthalein and calcium hydroxide. The contents of the flask are shaken and allowed to stand for 30 minutes, with occasional shaking; the solution should turn pink. At the end of the 30 minutes the solution is filtered through a fine filter paper into a test tube, and the tube is stoppered. If a test for protein shows that the filtrate still contains protein, more mercuric chloride is added and filtration is repeated. A solid rubber stopper is fitted from below into the bottom of a 30 cc. porous glass filter (Jena or pyrex glass filter, size 2, porosity 1G4) that has been dried and weighed. Approximately 20 cc. of freshly filtered uranium–zinc acetate reagent are pipetted to the filter standing on the rubber stopper. The reagent should be shaken frequently preceding use to assure saturation. Exactly 2 cc. of urine filtrate are pipetted to the reagent in the filter and the mixture is stirred with a small glass rod until a precipitate appears and for several minutes longer. The stirring rod is withdrawn and rinsed with 3 to 5 cc. of uranium–zinc acetate reagent during withdrawal. The filter is covered with a watch glass and set aside at room temperature for 1 hour. The rubber stopper is removed, the filter placed in a suction flask, and suction is applied. After the reagent has been filtered off, the precipitate is washed 5 times, each time with 2 cc. of the alcohol saturated with the triple salt, which should be filtered before use. The sides of the filter should be carefully washed down; if the washing is delayed, it becomes more difficult. Finally, the precipitate is washed twice, each time with 5 cc. of ether. Suction is continued until the precipitate is thoroughly dry. The filter is then placed in a desiccator over calcium chloride for 30 minutes and weighed.

A blank is run on a volume of distilled water equal to that of urine, and reagents.

The procedure yields accurate results if the 2 cc. urine sample analyzed contains between 1 and 10 mg. of sodium.

Calculation.

$$\text{Gm. Na per 100 cc. urine} = \frac{1.495(\text{Gm. precipitate} - \text{Gm. blank})}{s}$$

where 1.495 is per cent content of Na in precipitate, and s is the cubic centimeters of urine in sample.

$$\text{mEq. Na per L. urine} = \frac{650(\text{Gm. precipitate} - \text{Gm. blank})}{s}$$

Procedure for Urine Low in Sodium Content. Urines low in sodium are the result of low salt diets or of certain types of diuresis (page 232). When the urine contains less than 1 mg. of sodium in 2 cc., the above procedure is modified so as to concentrate the urine. For urine containing between 0.5 and 0.1 mg. of sodium per cubic centimeter, a volume of urine filtrate not exceeding 10 cc. containing between 1 and 3 mg. of sodium is pipetted to a small evaporating dish or beaker. Concentrated hydrochloric acid is added a drop at a time until the urine turns acid, and the sample is evaporated down to approximately 2 cc. If it is evaporated to dryness, 2 cc. of water should be added. The sample is then transferred quantitatively to the reagent in the weighed filter, as in the unmodified procedure, except that approximately 15 cc. of reagent are placed in the filter instead of 20 cc. The beaker is rinsed with 0.5 cc. of water, and then twice with reagent, 3 cc. each time. If a precipitate crystallizes out because of evaporation of the urine, transfer to the filter is made as usual; all the precipitate is washed into the reagent and stirring is continued a little longer. Even the slimy residue which may remain after evaporation is transferred directly to the glass filter. The solution in the filter is stirred, as described above, and the procedure is continued as above.

DETERMINATION OF SODIUM IN SERUM (66c)

Reagents. The same reagents are used as for determination of sodium in urine.

1 cc. of serum is pipetted into a thick-walled pyrex test tube, 200 × 25 mm., and a small quartz crystal, 1 cc. 4 *N* sulfuric acid, and 0.5 cc. concentrated nitric acid are added. Digestion is carried out in the usual way (43c). When charring appears, the flame is

removed, a few drops of superoxol or concentrated nitric acid are carefully added down the side of the tube, and digestion is resumed. If the solution does not clear, the addition of hydrogen peroxide or nitric acid and heating is repeated. Heating is continued for a few minutes after the solution has cleared, the tube is then cooled, and 4.5 drops of water are added. The contents are poured quantitatively into approximately 15 cc. freshly filtered uranium–zinc acetate reagent in a glass filter which has been previously weighed and stoppered, as described in the procedure for urinary sodium determination. The contents of the test tube are rinsed 3 times to the filter with 0.5 cc. water each time, and finally twice with 3 cc. reagent each time. The solution in the filter is stirred until a precipitate appears and for several minutes longer. The stirring rod is withdrawn and rinsed with 3 cc. of reagent during withdrawal. The remaining steps in the procedure and the calculation are the same as in the procedure for urine.

TABLE 40

Changes Observed in the Plasma of a Patient with Addison's Disease

Date, 1932	Na, mEq./L.	K, mEq./L.	Cl, mEq./L.	HCO_3^-, mEq./L.	N.P.N., mg./100 cc.	Remarks
July 19	123.3	5.3	88.6	21.8	39.0	Shortly after admission; critically ill; no therapy
July 26	107.8	7.1	72.7	21.5	45.0	
Aug. 2	133.0	5.1	93.8	27.5	20.6	After treatment
Nov. 14	139.9	4.6	107.3	24.3	20.0	Continuous therapy; patient up and about at home

From Loeb (66d).

The average normal concentrations of cations in plasma are:

Element	Mg./100 cc.	or	mEq./L.
Na	317		138
K	20		5
Ca	10		5
Mg	5		2

The clinical significance of decreased serum levels of sodium and potassium in acute dehydration will be found on pages 232 and 239.

An abnormal concentration of sodium and potassium in plasma is also an important biochemical sign of hypoadrenocorticism (page 458). Table 40 illustrates this.

IODINE

It has long been known that iodine is essential for production of thyroid hormone (67). Lacking an adequate supply of iodine, the anatomic structure and functional efficiency of the thyroid gland begin to suffer. When ingested iodine reaches the circulation, the thyroid retains the bulk of it. This gland under normal conditions contains about 20 per cent of the entire supply of iodine in the body, or 2 mg. per gram of dried gland. It is generally assumed that iodine aids the storage of colloid, the active principle of which consists of diiodotyrosine and thyroxine. These are linked together to form a peptide, which combines with other amino acids and is built up into thyroglobulin (colloid) (68b). Thyroxine has been synthesized as tetraiodothyronine. Thyroxine, as well as the colloid hormone, increases intracellular respiration and exerts an enormously stimulating effect on the oxygen consumption of tissue, with a corresponding rise in the basal metabolic rate.

The study of the metabolic fate of iodine is relatively new. For the clinician such studies are primarily interesting insofar as they attempt to measure thyroid function through the metabolism of iodine. Efforts have been made to use the iodine content of blood as an index of the thyroid gland's activity. This method can be useful, despite the wide normal range of the blood iodine level (4–32 γ), provided only levels which fall outside this range are considered as abnormal. Indeed, most workers have found a frequent correlation, although by no means an absolute parallelism, between the blood level of iodine and the functional activity of the thyroid gland (69). One serious drawback, however, is conceded by all workers: iodine levels within the normal range have been found in about a fourth of the patients with unmistakable, clinical signs of hyperthyroidism. Whether the results are less equivocal in hypothyroid patients is still a matter of discussion.

Elmer (70) suggests use of an iodine loading or tolerance test, as devised by Watson (71). The test ascertains the blood iodine curve by determining the iodine level in blood samples taken at 30 minute intervals for 2½ hours after oral administration of 6 minims or 0.4 cc. of Lugol's solution, the equivalent of 37 mg. of iodine. As determined in nonthyrotoxic patients, the curve normally rises to 80 micrograms or more per hundred cubic centimeters 30 minutes after ingestion of the test dose, and remains considerably above the fasting level during the remaining 2 hours of the test period. In hyperthyroid patients the curve is rather flat, failing to reach the normal maximum of 80 micrograms. Little is known of the response of hypothyroid patients to this test.

In children, these tests are not particularly useful. Fashena (69) has shown that age is not one of the variables affecting blood iodine concentration, except in the newborn. The normal average concentration in children 1 to 13 years old is 6.6 micrograms. Concentrations which may be safely regarded as abnormal are found only in conditions which are obvious clinically. The 10 to 15 cc. of blood for a single determination which the available "micromethods" (72,73) require make the test somewhat impracticable in small children, precluding the series of blood samples needed to determine a tolerance curve. To overcome these obstacles, Talbot *et al.* (74,75) have devised a colorimetric method which requires only 4 cc. of serum; only protein-bound iodine is determined by this method. To quote the authors, the iodine blood test in this form gives results which are "a direct index of the thyroid activity." Although it is a difficult method technically, determination of protein-bound iodine in serum may eventually prove to be a better test of thyroid function in small children than determination of the basal metabolic rate.

WATER

In the organism water is constantly on the move—from the alimentary tract into the blood stream, into the tissues, into the water depots, back again into the blood, and finally out of the organism by renal or extrarenal elimination. This movement of water is so well balanced that the water content of the body as a whole is maintained at a constant level. As conceived by Gamble (76), the mechanism of water balance rests on volume control in the several body fluid compartments. The localization and characteristic composition of the body fluids may be summarized as follows:

Intracellular fluid	Extracellular fluid	
(except in erythrocytes, renal epithelium, and special secretory cells of intestinal tract (77))	Low potassium, high sodium and chloride content	
	Vascular compartment	Interstital compartment
High protein, potassium, and phosphate content; sodium or chloride lacking	Holds protein-rich plasma	Holds ultrafitrate of plasma; protein content minimal

Fluid in the interstitial compartment changes more easily than in the other two compartments, water being taken in and let out continuously to conserve the volume in the vascular compartment and within the cells. When it becomes necessary, the volume of both interstitial and plasma water changes in order to protect the volume of intracellular fluid, which is the last to be affected.

Water movement and water content must always be considered in connection with the mineral constituents making up the electrolyte patterns characteristic of each compartment (78). Conversely, a changed rate of potassium or sodium movement also brings a change in the rate of water movement (79). The regulation of water balance and water exchange is under the control of a hypothalamic nerve center, and of thyroid, posterior pituitary, and adrenocortical hormones.

There are various methods for testing water metabolism. The facts and concepts just enumerated should facilitate a rational approach in considering these methods.

The relation of water content to concentration of solutes (salts and organic constituents) is less constant in the young organism than in that of the adult. The composition of the body fluids, the allocation of water between the several fluid compartments and the rates of turnover differ significantly with age (80). As a rule, the younger the child, the greater is the water content of the organism and the higher the rate of turnover (physiologic hydration). Some of these differences between the infant and the adult may be seen in Table 41. It has been demonstrated that the tissues of muscle and skin in young individuals have a particularly great affinity for water (84), and the tendency, observed throughout childhood, to retain water more easily is not due to a relative deficiency in renal function.

In the brief survey that follows, the available methods for test-

TABLE 41
Data on Body Water in Infants and Adults

Body water*	In infants	In adults
Body water content (81)	72% of body weight	60–66% of body weight
Plasma water content (82)	92–94%	91–92%
Extracellular water (83)	40% of body weight	20% of body weight
Incoming water (84)	158–172 Gm./Kg. body weight	41.4 Gm./Kg. body weight
Water ingested (84)	140–150 Gm./Kg. body weight	35 Gm./Kg. body weight
Water of combustion (84)	18–22 Gm./Kg. body weight	6.4 Gm./Kg. body weight

* Numbers in parentheses are reference numbers.

ing water balance and for measuring changes in the distribution of fluids among the various compartments of the body are outlined, and only procedures practicable as diagnostic aids are described in detail.

1. Changes in total body water content occurring from day to day can be estimated from changes in body weight. Although usually referable to variations in the volume of extracellular fluid, these changes in body weight merely indicate progressive accumulation or loss of body fluids.

2. To be complete, water balance studies must determine all the water available to the body (exogenous and endogenous) and all the water excreted (urine, stool, evaporation). Not every one of these items need be determined directly; a complete balance can be obtained by calculation, if certain data are known (85). These are: (*1*) Initial weight of subject. (*2*) Food ingested: (*a*) total weight, (*b*) water content, (*c*) protein content, (*d*) fat content, and (*e*) carbohydrate content. (*3*) Weight of total water ingested. (*4*) Urine: (*a*) total weight, (*b*) water content, (*c*) nitrogen content. (*5*) Stool: (a) total weight, (*b*) water content, and (*c*) nitrogen content. (*6*) Final weight of subject.

Levine and co-workers (86) have adapted the procedure for infants and have obtained the first complete information on the physiology of water balance in infancy. In view of the elaborate procedure, determination of water balance can obviously only be made use of in special investigations.

3. Extracellular water, i.e., the functional unit of vascular and interstitial fluid, can be measured, at least approximately, by dilution methods (87a,87b). Certain substances which are normally not present in the body, when brought into the circulation resemble chloride in their behavior—they are not diffusible, are not metabolized, are excreted slowly, and remain largely in the extracellular fluids. One of these substances is sodium sulfocyanate. From its concentration in plasma 1 or 2 hours after it is injected intravenously, one can calculate the amount of water that was available to dissolve the selected test dose; this "available fluid" provides the clue to the approximate volume of the total extracellular fluid. The method has been adopted for use in children, but it needs further perfecting. Radioactive sodium has also been successfully used to measure the volume of extracellular water.

4. Another method determines the balance of sodium, the dominant extracellular ion. According to Gamble (76), this can be determined approximately by measuring the urinary sodium excretion (page 223). Negative balances are indicated when sodium excretion is far in excess of the normal range; they signify depletion of extracellular fluid, i.e., dehydration. Reduced excretion of sodium, on the other hand, indicates a tendency to retain water in the extracellular spaces.

5. The intradermal test of Aldrich and McClure (100), which uses the skin as the test organ, provides information on the state of interstitial hydration. The significance and application of this test are discussed on page 237.

6. The state of vascular or plasma fluid can be determined by one of the dilution methods, using substances which remain in the vascular compartment when they are injected intravenously. A practicable procedure, modified for use in children, is described on page 235.

7. To test the functional ability of the body's water reservoirs, Wilder (88) examined the diuretic response of a child with glycogen storage disease to an intravenously administered dose of excess water. The patient excreted the administered dose of water within 1 hour, in contrast to normal individuals, in whom the diuretic response begins later and persists for more than 2 hours (79). Wilder expresses the opinion that the rapidity of response in his patient might be the result of glycogen infiltration of the liver cells which blocks the mechanism normally permitting an overflow of water to be harbored temporarily in the liver (89). Considering the many factors which determine the rate of diuresis, and the variety of organs serving as water depots, the results of such a "liver water storage test" may be variously interpreted.

8. Here, too, should be mentioned the "pitressin test" (page 393), which uses the response to an artificially induced disturbance in the body fluid relationships for the diagnosis of epilepsy, and the "water test" for the diagnosis of adrenocortical deficiency (page 460).

Clinically considered, water metabolism tests are particularly useful in revealing pathologic translocation of water when the clinical signs of dehydration or edema are still slight. In the older child, for example, 5 to 6 pounds of edematous interstitial fluid may accumulate before its presence can be detected by palpation (pitting), or inspection (77); and dehydration does not become significantly apparent until 8 to 12 per cent of the body weight are lost (104a,b).

It has been recognized (80,90) that *dehydration* may result from (*1*) excessive loss of water by vomiting, diarrhea, sweating, or hemorrhage; (*2*) restriction of water intake; and (*3*) loss of extracellular electrolytes, as a result of burns, surgical shock, or adrenal insufficiency. Characteristic laboratory findings in dehydration are (*1*) decreased volume of available water (page 231); (*2*) hyperproteinemia (page 165); (*3*) increased hematocrit values, i.e., increase in the relative red blood cell volume; (*4*) increased potassium–sodium ratio in the urine in extreme dehydration; (*5*) abnormal electrolyte concentration in plasma (*a*) when water loss is due to vomiting, chloride being reduced, sodium normal or decreased, and sodium bicarbonate increased; (*b*) when water loss is due to diarrhea, chloride being increased, sodium normal or slightly increased, and sodium bicarbonate decreased.

In acute dehydration with starvation some of these determinations serve as a basis for a choice of the best form of parenteral fluid therapy. Butler and co-workers have worked out a method of appraisal, which is outlined in detail on page 239.

According to McQuarrie (77), *edema* may be due to (*1*) depletion of plasma proteins, as in malnutrition, nephrosis, or hepatic insufficiency; (*2*) increased venous pressure, as in cardiac decompensation or chronic renal disease; (*3*) increased capillary permeability, resulting from toxic or other injury, as in acute renal disease, infectious disease such as scarlet fever and diphtheria, or allergic conditions. The characteristic laboratory findings are (*1*) increased volume of available fluid (page 231); (*2*) normal plasma volume; (*3*) hypoproteinemia (page 165); (*4*) decreased hematocrit values, i.e., decrease in the relative red blood cell volume (page 484); (*5*) decreased elasticity of tissue (page 237).

PLASMA VOLUME DETERMINATION

Unlike corpuscular volume, which varies widely, plasma volume remains almost constant under normal conditions, being so maintained by the proper rates of intracompartmental water exchanges and of diuresis. Prolonged failure of this mechanism leads to changes in the plasma volume (page 229).

Clinical measurement of plasma volume is accomplished by indirect or dilution methods. Certain dyes, when injected into the blood stream, remain in the plasma long enough to become uniformly mixed with the plasma fluid. These dyes do not enter the red cells, are not excreted by the kidneys, and are removed from the circulating blood (by phagocytosis in the reticuloendothelial system) so slowly that 20 to 30 minutes after their injection only minute amounts have escaped into the tissues. As a result, the plasma volume may be estimated from the amount of dye injected and its dilution by the circulating blood. The dye dilution method was introduced by Keith, Rowntree, and Geraghty (91).

The carbon monoxide method (92), another indirect method, has now been largely replaced by the dye method for clinical purposes.

PEDIATRIC CONSIDERATIONS

Normal standards of the relation between plasma volume, body size, and age have been established, and the method made more accurate by substituting electrocolorimetry for ordinary colorimetry. The method requires 5 blood samples of at least 4 cc. each, which is certainly a drawback to its use in infants and small children. A suggested micromethod (93), by which determinations can be made on 0.05 to 0.1 cc. samples of serum, has not yet been tried out on any large scale. The results of the carbon monoxide method, which has also been used in children (94a,b), are in line with those obtained by the dye method.

PROCEDURE

The method described is that of Gibson and Evans (95a) and Gibson and Evelyn (95b), as applied to children by Brines, Gibson, and Kunkel (96).

Apparatus. (*1*) Electrocolorimeter. (*2*) Hematocrit tubes. (*3*) 5 or 10 cc. syringes, with graduated plungers and barrels. (*4*) Centrifuge tubes coated with wax or paraffin.

Reagents. Evans blue (T-1824), 0.1 or 0.25 per cent solution in distilled water, filtered through sintered glass filter. Place the solution in neutral glass ampules of about 11 cc. capacity, seal, and autoclave at 121 C. for 20 minutes. The reagent is available commercially.

The determination is made in the morning with the child in a fasting state. Depending on the child's age, 4 to 8 cc. of blood are withdrawn from a cubital vein in older children, or from a jugular vein in small children. This serves as the dye-free sample and for hematocrit readings. Then the dye solution is slowly injected through the needle which has been left in place, 30 to 40 seconds being taken for injection. The amount of solution to be injected is 1 cc. of the 0.1 per cent solution in infants, 2 cc. of the 0.25 per cent solution in small children, and the adult dose of 10 cc. of the 0.1 per cent solution in older children. To make certain that all the dye is delivered, the syringe is twice rinsed with blood, provided it can be done without damaging the vein and resultant loss of dye into the tissues. In the course of the following 30 minutes, starting 10 minutes after the beginning of the injection, 4 blood samples are taken from the corresponding vein of the other side at intervals of 4 to 5 minutes. A fresh syringe must be used for each sample, but the needle may be left in place; clotting is prevented by injecting small amounts of saline between withdrawals. Each sample should be large enough to yield a little over 1 cc. of serum. In infants the total quantity of blood withdrawn will amount to about 20 cc.; in small children, to about 25 cc.; and in older children, to about 40 cc. Care must be taken to prevent hemolysis, as even slight degrees of hemolytic discoloration interfere with the determination.

The concentration of Evans blue in all 4 blood samples is determined electrocolorimetrically on the serum. Extreme variations in plasma volume are not revealed by measurement with the Duboscq colorimeter. A hematocrit reading is taken on at least 3 of the samples; if the whole amount of each sample is needed for colorimetry, 3 separate "hematocrit samples" are taken—at the beginning, the middle, and the end of the 30 minute period—and the readings are used for calculating dye dilution in the dye samples withdrawn at approximately corresponding times.

The author's instructions for determinations with the Evelyn electromicrocolorimeter are as follows:

> The same absorption cell is employed for readings on all dyed samples, provided it is well drained with a fine bulb pipet between samples. A separate cell should be used for the dye-free sample. About 1 cc. of serum is needed to fill the cell to the standard depth of 100 mm. With smaller quantities of serum a spectrophotometer is needed, using absorption cells of 5 mm. depth. Filter no. 620 is inserted for all determinations. With the cell containing the dye-free serum sample, the galvanometer is adjusted to read 100, the center setting aperture is racked into place, and the exact value of the center setting is noted. Then the dye samples are read in turn, the center setting being kept constant throughout. When the readings for the entire series have been made, the dye-free sample is again read and should check at the initial reading of 100.

Calculations. The galvanometer readings obtained for each dyed serum sample are recorded, and plotted against time on coordinate paper. The disappearance slope is extrapolated to intersect the ordinate, as illustrated in Figure 32. This extrapolated value is taken as the galvanometer reading G, to be used in the formula:

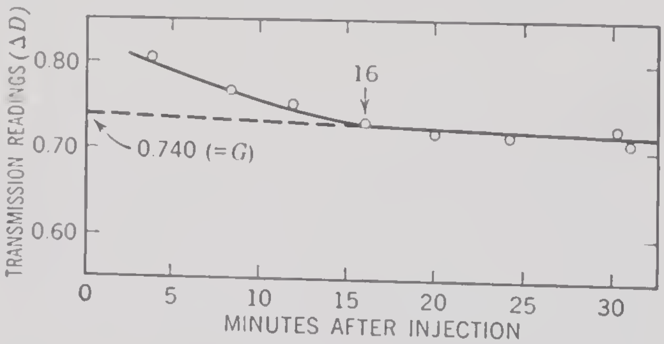

Fig. 32. Method of extrapolation of the disappearance slope to obtain the value of G, and thereby of L. on which the calculation of plasma volume is based. From Gibson and Evans (95a).

$$C = L/K \text{ (formula 1)}$$

where C is concentration of the dye in mg. per 100 cc. of serum, L is $2 - \log G$, and K is a constant the value of which must be determined for each new lot of dye by making a number of measurements of L for various known values of C.

The dye solution is standardized by determining the corresponding K values for dye solutions in serum, using cells 10 mm. deep and filter no. 620. A series of standards is prepared, in concentrations of 1.0, 0.333, 0.25, and 0.2 mg. per 100 cc. of serum for the 0.1 per cent dye solution, and in concentrations of 2.5, 1.5, 1.0, 0.75, and 0.6 mg. for the 0.25 per cent dye solution. The average of the values obtained for the individual standards is taken as the K value for the solution employed ($K = L/C$).

$$\text{Plasma volume, cc.} = M/C = MK/L \quad \text{(formula 2)}$$

where M is the milligrams of dye injected.

Since K is known as far as the same lot of dye is used, the calculation is made from the extrapolated G value by substituting the corresponding L value in formula 2.

$$\text{Total blood volume, cc.} = \frac{\text{plasma volume}}{100 - \%\text{ of cells}} \times 100$$

$$\text{Red cell volume, cc.} = \text{total blood volume} - \text{plasma volume}$$

The figures thus obtained are incorrect, since the blood in the smaller vessels and capillaries contains fewer cells per unit volume than that in larger vessels. It has not yet been established, however, whether a correction by the factor 0.75 eliminates the error incurred, and the uncorrected figures may be used for all practical purposes.

INTERPRETATION

Height is the correlative factor of choice in the determination of normal plasma and blood volumes in children. The chart of Brines, Gibson, and Kunkel (96) shows normal values of plasma and total blood volumes referred to height (Fig. 33). Actual figures which fall within plus or minus 15 per cent of the values in the chart may be considered normal, provided the child's physical measurements fall within the accepted anthropometric norms (page 67).

Plasma volumes in children of abnormal physique are far outside the normal range.

Decreased plasma volume occurs after severe hemorrhage and burns, in surgical shock, hypothyroidism, acute crises in Addison's disease, severe dehydration of diarrhea or vomiting, and sometimes in the edema of cardiac decompensation.

Increased plasma volume occurs in hyperthyroidism, hepatic cirrhosis, congestive heart failure, and in pernicious anemia.

So long as the red blood cell volume remains fairly constant, variations in total blood and plasma volumes are directly correlated. But if the red blood cell volume also changes, the total blood volume will depend on the combined effect of the changes in cell and plasma volumes (97).

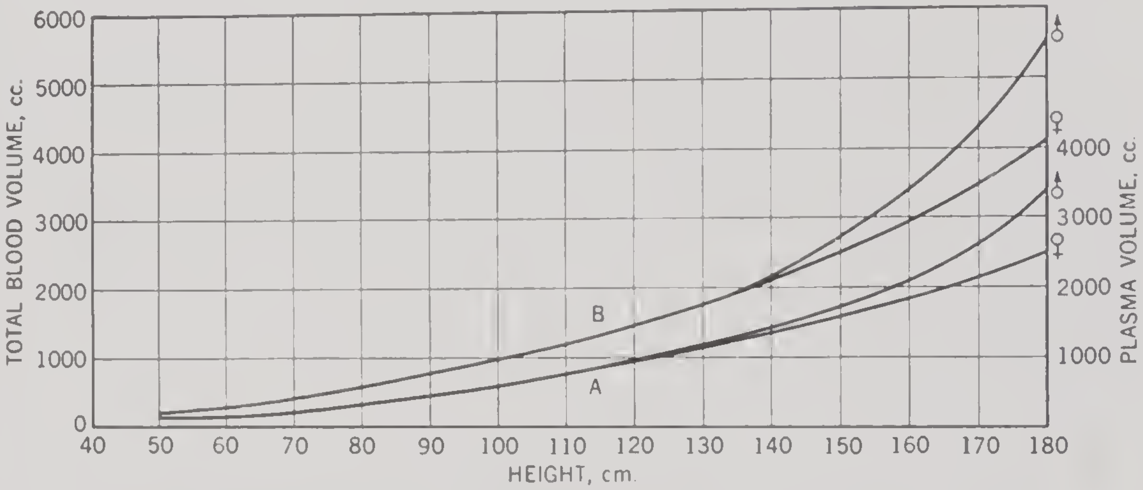

Fig. 33. Nomogram for predicting the normal volume of plasma and of total blood in infants and children. A: Plasma volume. B: Total blood volume. From Brines, Gibson, and Kunkel (96).

INTRADERMAL SALINE TEST

The method has been devised by McClure and Aldrich (100). The test measures the time required for a small amount of isotonic saline solution, injected intradermally, to be absorbed. Apparently, absorption time depends on the state of hydration of the skin, for in various conditions associated with superhydration or dehydration the time differs considerably from that observed in normal subjects. Glucose solution has been reported to yield substantially the same results as the saline solution (98). Various theories have been advanced to explain the differences in absorption time. The opinion prevailing at present is that the speed of absorption depends on local mechanical factors, such as elasticity, rather than on the avidity of interstitial tissue for water (99).

PEDIATRIC CONSIDERATIONS

Age is one of the factors influencing absorption time. Normal standard values for children of all ages therefore had to be established before test results could be evaluated. Since the standards have become available, the intradermal saline test has come into wide use as a test of the imminence, intensity, or subsidence of an edematous condition, and for states of dehydration. Such information is especially valuable when there are neither visual nor palpable signs of any change in the hydration of the skin.

PROCEDURE

With a 27 gage needle, 2 wheals are produced on the upper half of the forearm 2 cm. apart by the intradermal injection of 0.2 cc. of an 0.8 per cent solution of sodium chloride. To avoid linkage during the injection, the needle is inserted in such a way that the beveled edge can be seen faintly in the skin. The time it takes the blebs to disappear is measured from the moment the first bleb was made to the moment when the valley between the two blebs can no longer be felt.

INTERPRETATION

Table 42 gives the normal absorption times in children. Abnormally shortened times, i.e., rapid disappearance of blebs, indicates a state of pathologic hydration, whatever its cause may be. In addition to cardiac, nephritic, toxic, or infectious edemas, any number

TABLE 42
Normal Rates of Absorption (McClure-Aldrich Test)

Age*	Normal range, minutes	Average, minutes
Newborn (99)		
Before physiologic loss of weight or after its restoration.......	6–16	10.4
During physiologic dehydration	18–46	26.3
Infants, 3 weeks–1 year (101)....	18–42	29
Children (101)		
1–5 years....................	21–50	34
6–13 years..................	40–78	52
Children over 1 year (100)......	—	60
Adults (100)..................	60–80	70

* Numbers in parentheses are reference numbers.

of conditions may show an abnormally rapid absorption time, for example, cardiac decompensation, toxic scarlet fever, diphtheria, lobar pneumonia, infantile eczema. In the newborn, except during the period of dehydration, and during early childhood, a relative superhydration is normal (page 229), which explains why absorption time increases progressively with age.

Abnormally lengthened absorption times, i.e., slow disappearance of blebs, indicate dehydration. Clinical examples may be found in the physiologic dehydration of the newborn and of premature infants (102), and the dehydration in acute nutritional disorders resulting in diarrhea, in dysentery, and in postoperative states (103).

Determination of State of Dehydration and Appraisal for Parenteral Repair Therapy

Although clinical examination and a detailed history are the basis for any appraisal when acute dehydration with starvation occurs in a child, chemical blood analysis is also essential—at first in order to determine whether the condition is acidotic or alkalotic, and later as a check on therapeutic results.

The system devised by Butler and Talbot (104a,104b) for chemical appraisal of dehydration and institution of parenteral repair therapy includes: (*1*) Determination of the approximate weight loss; this serves as a basis for calculating the total fluid loss which must be repaired. (*2*) Estimation of serum proteins (page 160) and hemoglobin; the inferences which may be drawn from these figures are given in Table 42a. (*3*) Nonprotein nitrogen may be determined (page 161), as a check on renal function. (*4*) Determination of serum concentration of (*a*) chlorides (page 462), (*b*) sodium (page 225), or (*c*) bicarbonate, which provide information on the character and extent of water and electrolyte depletion in the compartments. These analyses permit differentiation between acidotic and alkalotic dehydration, metabolic or respiratory. Metabolic acidosis, for instance, is indicated by a low bicarbonate content and/or sodium deficit, with normal or subnormal chloride levels. A full description of the changes in the electrolyte pattern associated with acidosis and alkalosis may be found in Gamble's monograph (76).

Estimation of plasma bicarbonate (alkali reserve) is carried out according to the procedures used in adults (43d). The pediatrician should be mindful of the differences in normal values between infants and adults (105). These range:

Breast-fed infants	51–52 volume per cent CO_2
Bottle-fed infants	45–50 volume per cent CO_2
Adults	55–60 volume per cent CO_2

The analytic data of these tests serve as a guide for the selection and dosage of the required repair fluids. Solutions of sodium chloride, bicarbonate, or lactate, administered parenterally, provide for extracellular needs, replenishing water and electrolytes in the vascular and interstitial spaces. Therapy of intracellular depletion has been, until recently, limited to parenteral administration of blood, plasma, and amino acids. However, direct replacement of the dominant intracellular ion, potassium, has also become feasible since Govan and Darrow (106) have shown that solutions of potassium chloride can be administered without great danger of complications and with striking therapeutic effect.

TABLE 42A
Estimation of Extracellular Parenteral Needs, Over and Above Maintenance Needs of Two Dehydrated Acidotic Patients

Fluid loss and repair needs	Infant, 5 Kg. wt.	Adult, 60 Kg. wt.
Fluid loss		
Weight before dehydration	5,000 Gm.	60,000 Gm.
Weight during dehydration	4,400 Gm.	54,000 Gm.
Approximate fluid loss	600 cc.	6,000 cc.
Extracellular loss (½ fluid loss)	300 cc.	3,000 cc.
Intracellular loss (½ fluid loss)	300 cc.	3,000 cc.
Provision of repair needs		
1 part 7 *M* sodium bicarbonate (or 7 *M* sodium lactate) and 2 parts physiologic saline solution (for extracellular loss and acidosis)	300 cc.	3,000 cc.
Serum (for protein depletion)*	100 cc.	800 cc.
Whole blood (for hemoglobin and protein depletion)†	100 cc.	500 cc.

From Butler and Talbot (104b).

* To be given if serum protein level is low before or during parenteral therapy.

† To be given if red cell count or hemoglobin level and serum protein level are low before or during parenteral therapy.

Table 42A summarizes the directions for parenteral repair therapy of acute dehydration, as given by Butler and Talbot. It appears from the table, that acidotic dehydration of diarrhea, for instance, calls for combined administration of bicarbonate (or lactate) and saline solution; alkalotic dehydration, the result of vomiting, requires use of saline solution. The amounts may also be calculated from the table.

Data obtained 24 hours after treatment is started enable the physician to appraise the relative efficacy of the treatment instituted, and provide an indication as to whether such therapy is to be continued or whether the patient may be put on daily maintenance doses (104a).

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CHAPTER VIII

Vitamin Nutrition Tests

In Jolliffe's (1) words: "A nutritional inadequacy begins the instant that adequate amounts of an essential nutrient fail to reach the internal environment. This results, after varying periods, in malnutrition, the successive stages of which are represented by (*a*) tissue depletion, (*b*) biochemical lesions, (*c*) altered function, and finally by (*d*) anatomical lesions. . . . The clinical recognition of these stages of malnutrition, as a rule, follows the reverse order, i.e., anatomical 'lesions,' altered function, biochemical 'lesions,' and tissue replenishment." The importance and truth of the above statements with regard to vitamin deficiencies and their detection by tests will become apparent in the discussion that follows.

VITAMIN A

Man's most important sources of vitamin A are compounds belonging to the class of carotenoids, which are present in all green or yellow parts of vegetables. One group, the precursors of vitamin A, consists mainly of the carotenes and is often termed "provitamins A"; the other group includes xanthophyll (lutein) and other phytoxanthins. Vitamin A itself has never been found in plants, and the animal organism must build it up from the provitamins. It is stored in the liver, fish livers containing the greatest quantities of this vitamin. According to Rosenberg (2), nine different provitamins and two forms of vitamin A are known to occur naturally.

Chemically, all provitamins A are characterized by an aliphatic chain containing a continuous system of ten conjugated double both ends of the chain (2a). Vitamin A has a similar structure, but it contains half as many carbon atoms as carotene, i.e., a continuous bonds differing from each other in the structure of the groups at series of five conjugated double bonds, and different side chains. None of the provitamins have as yet been synthesized, nor have attempts to synthesize vitamin A yielded the pure substance. Both vitamin A and the provitamins are fat soluble but insoluble in water.

The international standard for vitamin A is the International Unit (I.U.), with 1 I.U. being equal to 1 U.S.P. Unit or 1.5 to 2 Sherman Units; 1 Cod Liver Oil Unit (C.L.O.) equals 208 I.U. or U.S.P. Units.

The International Unit represents a vitamin A potency which equals the potency of 0.6 microgram of beta-carotene, as judged by the biologic test on young rats which have ceased growing because of vitamin A deficiency. Under the specified conditions of the rat test, beta-carotene is converted into an equimolecular quantity of vitamin A. But, according to Rosenberg (2b), "an equality of an International Unit of vitamin A and of carotene can be claimed only under those conditions and is actually quite different for the metabolism of a normal growing organism."

Ingested provitamins A, as well as vitamin A, are absorbed by blood and lymph from the intestinal tract and transported to the body tissues chiefly in the form of ester compounds (3). Rate of absorption depends upon the amount of fat, bile, and pancreatic lipase present. Both the provitamins and vitamin A are removed from the plasma by the reticuloendothelial system, but only vitamin A is stored, chiefly in the liver (4); small amounts are also found in the adrenal cortex and the ovaries (2c). Although it is not yet known how provitamins are transformed into vitamin A, it seems probable that the liver is the site of the transformation. Vitamin A and its precursors are easily catabolized; the vitamin is not excreted in the urine, and the feces contain only what has escaped absorption.

Though the mechanism of vitamin A action is largely unknown, the physiologic importance for man of an adequate supply of vitamin A has been established (5a). In addition to being a growth vitamin, it prevents xerophthalmia and hardening of mucous membranes; the vitamin also plays a specific role in the regeneration of visual purple or rhodapsin (page 259), and probably in the reproductive function.

The daily vitamin A requirements, as postulated by the National Research Council (5b) are:

Age	Vitamin A, I.U.
Up to 1 year	1,500
1–7 years	2,000
7–10 years	3,500
10–13 years	4,500
Adults	5,000–6,000

Clinical signs of avitaminosis A appear only when the stores of the vitamin have become almost completely depleted; for this reason, clinical tests are needed by which incipient and advanced stages of deficiency may be detected. The following test methods provide such diagnostic help: (*1*) determination of vitamin A levels in the blood; (*2*) dark adaptation test; and (*3*) vitamin A absorption test.

ASSAY OF VITAMIN A AND CAROTENOIDS IN BLOOD

Determinations can be carried out on plasma or serum, since the corpuscles are almost devoid of these compounds. The analytic methods are based on color reactions with phenols, acids, or inorganic chlorides. Practically the only reaction utilized for assays of vitamin A in plasma is that of Carr and Price (6a), which produces a blue color when vitamin A is treated with antimony trichloride. Since the carotenoids which may be present in the plasma affect the intensity of the Carr-Price reaction, allowance must be made for the carotenoid concentration, which should be determined separately. No standardized preparation of vitamin A is available for this colorimetric test on plasma, so that the intensity of the Carr-Price reaction is expressed in terms of arbitrary units. To convert these units to International or U.S.P. Units which are biologic by definition requires the determination of a so-called "conversion factor," a procedure accepted for commercial use (6b). Similar technics for clinical assays are not yet being used.

Total carotenoids are usually determined spectrophotometrically or colorimetrically by comparison with standard dye solutions, and the results are expressed in terms of beta-carotene or of arbitrary units.

The blood vitamin A level of infants and children has been studied by the same methods as in adults. The electrocolorimetric determination is more accurate, and since it requires only 1 cc. of plasma or serum it is especially convenient for use in children. If an electrocolorimeter is not available, the procedure recommended by Claussen and McCoord may be used; it is simple to perform, but requires 3 cc. of plasma.

ELECTROCOLORIMETRIC ASSAY OF VITAMIN A AND CAROTENOIDS

The method described is that of May, Blackfan, McCreary, and Allen (7), and uses the Evelyn photoelectric colorimeter (8).

Reagents.

(*1*) 95 per cent alcohol.
(*2*) Purified petroleum benzine, U.S.P. (petroleum ether).
(*3*) 22 per cent solution of antimony trichloride.

Technic. Into a narrow-mouthed, glass-stoppered tube are transferred 1 cc. of plasma or serum and 2 cc. of 95 per cent ethyl alcohol. The mixture is shaken and 2 cc. of petroleum ether are added. The tube is then stoppered tightly, and shaken vigorously and continously for 10 minutes. A clear upper layer of petroleum ether extract is obtained by centrifugation; this is used in the further analysis.

Filter no. 440, i.e., with a maximum transmission for a wave length of 440 millimicrons, is placed in the microunit of the photoelectric colorimeter and the center setting of the instrument for 1 cc. of petroleum ether in a 1 cc. open type microabsorption cell is determined (8). A similar center setting is determined for 1 cc. of the antimony trichloride solution in chloroform. The center settings must be determined immediately before each analysis, and all operations must be performed quickly to ensure accuracy.

With filter in place and the instrument set at the center setting for petroleum ether, 1 cc. of the petroleum ether extract of the serum is measured into a 1 cc. open type cell in place in the cell holder. The cell is immediately wound into the colorimeter and the galvanometer reading is recorded. This reading (G_{440}) is used in calculating the total amount of carotenoids.

The cell with its contents is then carefully removed from the colorimeter without allowing any of the extract to spill. The petroleum ether is evaporated to dryness by gently blowing dry air into the cell while it is held partially immersed in water kept at 40 to 45 C; a petri dish with a filter paper in the bottom to keep the polished bottom of the cell from being scratched may be used. The outside of the cell is then wiped dry and polished with lens paper, and the cell is replaced in the cell holder. Filter no. 620 is put into the colorimeter and the galvanometer is set at the center setting for antimony trichloride reagent. When everything is ready 1 cc. of the reagent is pipetted into the cell and it is wound immediately into the colorimeter. The blue color attains its maximum intensity in a few seconds. The galvanometer reading, G_{620}, is taken at the point of maximum absorption of light, i.e, maximum color intensity.

Calculation. The results, as obtained in an Evelyn colorimeter, are expressed in terms of units, calculated as follows:

$$L = 2 - \log G$$

$$\text{Units per 100 cc. serum} = L \times \frac{2}{\text{cc. serum analyzed}} \times 100$$

Results for carotenoids are designed as "L440 units" and for vitamin A as "L620 units," indicating the maxima of the light band by which the color intensity is measured.

When both readings (G_{440} and G_{620}) are expressed in terms of units by means of the above equation, the value of L440 units, representing carotenoids, is converted into the equivalent value of L620 units by using the factor 0.11. The number of L620 units contributed by the carotenoids is subtracted from the total number of L620 units expressing the intensity of blue color formed by the addition of antimony trichloride, and the result is the value of L620 units of vitamin A.

Example. Analysis of 1 cc. of the petroleum ether extract from 1 cc. of serum yielded:

Readings: G440 = 71; G620 = 81
L values: L440 = 0.1487, and L620 = 0.0915
L440 units/100 cc. $= 0.1487 \times \frac{2}{1} \times 100 = 29.74$ (total carotenoids)
L620 units/100 cc. $= 0.0915 \times \frac{2}{1} \times 100 = 18.30$
L620 units/100 cc. from carotenoids $= 29.7 \times 0.11 = 3.3$
L620 units/100 cc. from vitamin A $= 18.3 - 3.3 = 15$

COLORIMETRIC DETERMINATION OF VITAMIN A AND CAROTENOIDS, ACCORDING TO CLAUSSEN AND McCOORD (9,9a,10)

Reagents.

(*1*) 95 per cent ethyl alcohol.
(*2*) Petroleum ether, U.S.P.

(*3*) Bichromate standard. Prepare a 0.2 per cent aqueous solution of potassium bichromate; this keeps for months. By diluting aliquots of this solution with water, a 0.02 per cent solution is obtained immediately before use. The diluted solution is used as the standard.

(*4*) Chloroform.

(*5*) 30 per cent solution of antimony trichloride in chloroform.

(*6*) Copper sulfate standard. 10 per cent aqueous solution of copper sulfate ($CuSO_4.5H_2O$).

Carotenoid Determination. 3 cc. of 95 per cent ethyl alcohol are added to 3 cc. of plasma or serum, the two are mixed, and 3 cc. of petroleum ether are added. The mixture is shaken at intervals for 10 minutes, and then centrifuged. An aliquot of the upper layer of petroleum ether is compared in the Dubosq microcolorimeter with the bichromate standard. When the sample is of exactly the same intensity of color as the standard, the original plasma is said to contain 100 units of carotenoid per hundred cubic centimeters.

Vitamin A Determination. 1 cc. of the petroleum ether extract of serum or plasma, already described, is transferred from the centrifuge tube in a small test tube and evaporated to dryness. Then 0.1 cc. chloroform and 1 cc. antimony reagent are added. The mixture is rapidly transferred to one cup of the Dubosq colorimeter with the plunger set at 10 mm., with the other cup of the colorimeter containing the solution of copper sulfate. Comparison must be made within 30 seconds after mixing. When the color of the extract mixture is exactly matched by that of the copper sulfate standard, the serum is said to contain 100 blue units of vitamin A per hundred cubic centimeters. Correction is made for the carotenoids of the serum by subtracting 2.7 blue units for each 100 units total carotenoids, as determined above.

INTERPRETATION

The average plasma concentrations of vitamin A and carotenoids in normal infants and children are listed in Table 43. Except for the neonatal period, vitamin A levels vary little with age. The carotenoids, however, do not rise to the average normal concentration until green vegetables and carrots are added to the diet, i.e., between the ages of 3 and 5 months. Both the vitamin A and the carotenoid levels are remarkably constant in the individual child.

TABLE 43
Normal Blood Concentration of Vitamin A and Carotenoids as Measured by Two Methods

Method of May, Blackfan, McCreary, and Allen (7)

Age	Vitamin A, L620 units per 100 cc. plasma	Age	Carotenoids, L440 units per 100 cc. plasma
0–1 mo.	8.1	0–6 mos.	5.6
2–12 mos.	11.9	7–24 mos.	51.3
1–6 yrs.	14.4	3–6 yrs.	41.7
7–12 yrs.	14.1	7–12 yrs.	39.5

Method of Claussen and McCoord (9)

Age	Vitamin A, blue units per 100 cc. plasma	Age	Carotenoids, units per 100 cc. plasma
Birth	12.8	Birth	20
0–6 mos.	23.8	18 mos.	100
6–24 mos.	19.9	—	—
2 yrs.	20.2	5 yrs. and over	90

Lower than normal levels of vitamin A in plasma are a sign that body stores are depleted; they aid the early clinical diagnosis of vitamin A deficiency (7). Values from 6.8 to 10 L620 units are obtained in mild deficiencies, while severe depletion is characterized by values ranging from 1 to 7 L620 units. Corresponding values in blue units are approximately 15 units in mild depletion, and 6.8 to 9.1 in severe depletion.

Conditions in which vitamin A concentration in the blood frequently drops below normal levels, and the probable causes for the drop are: (*1*) True hypovitaminosis, due to insufficient dietary intake of the vitamin. (*2*) Impaired intestinal absorption of the fat-soluble vitamin A in (*a*) celiac disease (7,10), due to intestinal hypomotility; (b) pancreatic fibrosis (7,10), due to lack of pancreatic enzymes; (*c*) hepatic disease (11), due to impaired bile secretion. (*3*) Diabetes; a small percentage of diabetic patients show subnormal concentrations of vitamin A (12), but the metabolic disorder causing it has not yet been defined. (*4*) Hypothyroidism (9), probably leading to inability to convert carotene into vitamin A. (*5*) Acute or chronic infectious diseases (7,13); what causes the decrease has not yet been clearly established.

Increased levels of vitamin A in plasma are generally found in the nephrotic syndrome, probably due to the liver's inability to store the vitamin (9).

According to Josephs (14), increasing the dietary intake of vitamin A beyond the optimum will not raise the vitamin A level in the blood above a normal maximum except for a few hours after administration.

As for the carotenoids, lower than normal concentrations in plasma are found in true hypovitaminosis A, in the celiac syndrome, and during the acute stage of many infections (13). Increased levels usually occur in conditions associated with hyperlipemia, as in nephrosis or hypothyroidism (9,14).

VITAMIN A ABSORPTION TEST

The method described is that of Chesney and McCoord (10). The vitamin A level in the blood is followed up for 12 hours after oral administration of a standard dose of the vitamin. Normally, the concentration in the serum is increased for several hours after ingestion of the test dose. Failure of the concentration to rise is a sign of impaired intestinal absorption of the vitamin. It seems probable that intestinal absorption of vitamin A is linked with that of fat, so when fat absorption is impaired, deficient absorption of vitamin A may also be expected.

PEDIATRIC CONSIDERATIONS

The test is invaluable, and is now commonly used, for the differentiation of vitamin A deficiencies in children due to a dietary lack of the vitamin and those caused by deficient intestinal absorption. The test also yields interesting information in various other conditions during childhood.

PROCEDURE

No vitamins except those contained in the normal diet should be given for a week prior to the test. The child must be free of fever for 2 to 3 weeks before the test, since acute or chronic infectious diseases interfere with vitamin A absorption.

The first blood sample is taken in the morning, before breakfast; the child is then given 0.2 cc. of oleum percomorphum per kilogram of body weight, by dropper for infants, by mouth otherwise. Blood specimens are then taken 3, 6, 9, and 12 hours thereafter. Diet is normal throughout the test period.

Vitamin A concentration in each of the blood samples is determined by one of the two methods already described.

INTERPRETATION

Normally, vitamin A concentration increases by more than 50 units, L620 or blue, following administration of the test dose. The range of the rise is from 50 to 230 units, with an average of 100 to 130 units. The peak occurs 3 to 5 hours after ingestion of the test dose; the level then declines and reaches the fasting value between 9 and 12 hours after ingestion. Age has no particular influence on the rate of absorption.

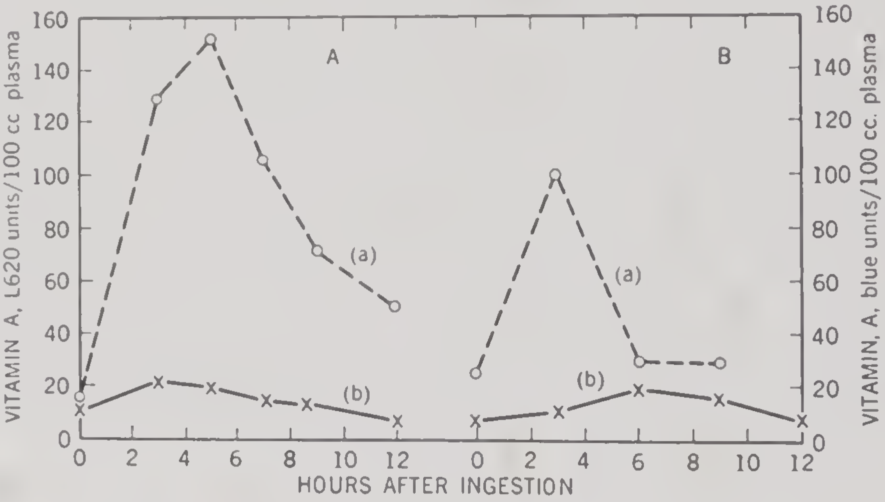


Fig. 34. Vitamin A absorption curves. A, adapted from May, Blackfan, McCreary, and Allen (7): (a) normal 21 month old child; (b) 21 month old child with celiac disease. B, from Di Sant'Agnese and Larkin (18): (a) normal 3 month old child; (b) 7 month old child with infantile eczema.

A rise of less than 50 units, or no rise at all, is considered an abnormal response, producing the "flat curve" (Fig. 34). The peak of such curves usually exceeds the fasting level by no more than 30 units; occasionally, flat curves also show a delayed rise. This failure to respond to a test dose of vitamin A is generally interpreted as the result of impaired intestinal absorption of the vitamin, particularly if the fasting level is normal.

Since impairment of other metabolic phases of the utilization and storage of the vitamin (page 250) must obviously also interfere with normal disposal of the loading dose, tolerance test would seem to be a more exact name than absorption test.

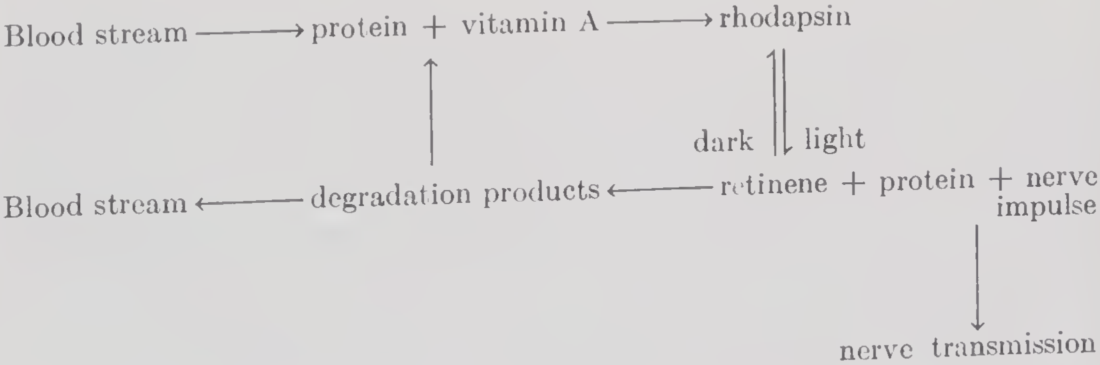
Flat curves have been obtained with this test in the following conditions in children: (*a*) Celiac disease (7,9,15,16); a flat curve is one of the diagnostic criteria in this condition. (*b*) Pancreatic fibrosis (7,9,16). (*c*) Obstructive jaundice, as in cirrhosis of the liver or atresia of the bile ducts (16). (*d*) Parenchymatous jaundice, as in catarrhal icterus (11,17). (*e*) Cretinism (7). (*f*) Ulcerative colitis (16). (*g*) Infantile eczema (18). (*h*) Acute or chronic infectious diseases, such as scarlet fever, measles, pneumonia, sinusitis, bronchiectasis, osteomyelitis, tuberculosis, rheumatic fever (7,19a), giardiasis, an infection with the parasite Giardia lamblia (19b). Two examples of a flat curve are given in Figure 34.

Pratt and Fahey (20) have recently suggested that the test might be simplified and thereby made more suitable for use in infants and young children. Only one blood sample is required; in infants up to the age of 6 months it is withdrawn 4 hours after administration of the test dose, in all other children at the end of 5 hours. A fasting blood sample is not required. Interpretation of the single vitamin A value obtained is similar to that in the original method. A rise of less than 50 L620 units above the average normal plasma concentration is considered abnormal. The average rise obtained by this method in celiac disease and other forms of the celiac syndrome is between 8 and 15 units.

DARK ADAPTATION TEST

Dark adaptation enables a person to see in dim light; decreased dark adaptation leads to night blindness or functional hemeralopia.

SCHEME I



Dark adaptation depends on the regeneration of the visual purple (rhodapsin) in the retina. Rhodapsin, a carotenoid albumin, bleaches out under the influence of light, forming retinene, and regenerates in the absence of light. Scheme I, which is drawn according to Rosenberg (2d), shows how vitamin A participates with rhodapsin in this retinal cycle.

The pigmented layers of the eye contain large amounts of vitamin A, essential for the synthesis of rhodapsin. An inadequate supply of this vitamin impedes regeneration of rhodapsin and thus reduces dark adaptation ability. This functional hemeralopia may well be the earliest sign of vitamin A deficiency (21).

Various photometers and methods have been developed for measuring dark adaptation in adults. These tests determine: (*1*) the rapidity with which clear vision is recovered in darkness after the retina has been exposed to bright light and thereby bleached, and (*2*) the minimum amount of subdued light that is visible to the dark-adapted eye, the so-called "brightness threshold."

Dark adaptation tests for assessing the vitamin A status of the body have been simplified by the discovery (22,23) that in dietary hemeralopia the rapidity of dark adaptation is practically unchanged and that the chief abnormality is a rise of the brightness threshold. It has thus become possible to confine the actual test to a determination of the threshold for vision in the dark, i.e., the final or equilibrium threshold.

PEDIATRIC CONSIDERATIONS

Older children can be tested for dark adaptation like adults. Young children and infants, however, cannot tell the moment they see light; in them the first reaction to light is recognized by the appearance of motor reflexes, such as movement of eyelids, facial muscles, and head. Thus, in the test procedure of Haig and Lewis (24), described below, the minimum intensity of light which elicits a movement of the head in the direction of the light is considered the infant's threshold for vision in the dark. These threshold determinations do not require fixation of the patient's eye in relation to the light stimulus. Another technic, also useful for testing infants, has been recommended by Friedrichsen and Edmund (25).

The dark adaptation test is almost as sensitive as the determination of plasma concentration of vitamin A for detecting vitamin A deficiency, but it is not as good as the vitamin A absorption test (26). Daily fluctuations in vitamin intake apparently affect dark adaptation far less than the plasma level of vitamin A. Some observations seem to indicate that impaired dark adaptation may be found in the presence of normal plasma levels of vitamin A.

PROCEDURE

The method described is that of Haig and Lewis (24). The adaptometer consists of two units—one containing battery, ammeter, and rheostat, the other a test light unit connected by a flexible cord to the first unit and so constructed as to fit into the operator's hand.

The light in the test unit emanates from a 3.8 volt flashlight bulb and passes through a system of photometric wedges, diaphragms, and filters. This arrangement enables the operator to vary the intensity of the light in steps of 0.3 log unit (1:2) through a range of just over 5 log units (1:100,000).

The brightness of the resulting light field for each setting of the wedge and filters is given in a table accompanying the apparatus. The brightness unit adopted is the micromicrolambert ($\mu\mu$L) expressed in logarithms. Thus, a threshold value of 2 (100 $\mu\mu$L) represents a response to an intensity of light 10 times weaker than a value of 3 (1,000 $\mu\mu$L) and 100 times weaker than a value of 4 (10,000 $\mu\mu$L).

If the patient is an infant or young child, a luminous pendant (such as used on electric light chains) is attached to the center of the child's forehead with adhesive tape and the child is placed on his back. The room is then darkened; after 30 minutes the test light unit, held in the operator's hand, is slowly moved from side to side through an arc of 180 degrees at about 10 cm. distance from the child's eyes. When the test light is made sufficiently bright, the child sees it in the periphery of his visual field and turns his head in the corresponding direction. This movement is clearly indicated by the motion of the luminous pendant, and is easily distinguished from random movements, after a little experience. The brightness needed to evoke any response is recorded.

The procedure for older children and adults is the same, except that the patient is asked to tell immediately when and from what direction the light is seen. The test light is moved over the entire visual field on both sides, the brightness of the stimulus is gradually increased and the light is occasionally turned off and on.

INTERPRETATION

The normal visual threshold, as determined by the adaptometer, ranges between 2.1 and 2.9 log units, depending on the subject's age (Table 44). This threshold remains unchanged in vitamin A deficiency, provided the daily intake is not extremely low. However, when the vitamin A content of the diet is less than 120 I.U. per day, the threshold increases about a hundredfold (Table 44). Values above 3.5 log units, under the above test procedure, should probably be considered as pathologic.

TABLE 44
Threshold for Vision in the Dark

Age and vitamin A nutrition	Mean value (log $\mu\mu$L)	Range (log $\mu\mu$L)
Normal		
Infants	2.9	2.4–3.3
Children and adults	2.1	1.6–2.5
Vitamin A deficiency		
Infants with daily intake of 135–200 I.U. for 3–6 mos.	2.8	2.5–3.0
Infants with daily intake of 60–120 I.U. for 3–6 mos.	4.7	—

After Lewis and Haig (24a).

It has been found that administration of large doses of vitamin A restores the threshold to normal within an hour.

VITAMIN B_1 (THIAMINE)

Vitamin B_1 or thiamine is present in many plants. The highest concentrations of thiamine are found in yeast and in the outside coat or bran of grains, particularly of rice. It is also present in animal tissue, especially in the liver, kidneys, muscle, and heart.

Vitamin B_1 is composed of a pyrimidine ring and a nitrogen-carbon-sulfur ring which contains pentavalent nitrogen (thiazole). The three forms of the vitamin that are of clinical interest are thiamine chloride (the commercially available salt of thiamine), thiamine pyrophosphate (the cocarboxylase which acts as coenzyme with carboxylase in the oxidation of pyruvic acid, a normal intermediary in the catabolism of sugar), and thiochrome (a fluorescent oxidation product of thiamine used in the determination of vitamin B_1 in body fluids).

The vitamin is also known as the antiberiberi factor, clinical and experimental studies of beriberi having shown that it is lack of this vitamin which affects the emotions, the tonus of the nervous system, physical endurance or muscle tone, and gastrointestinal and cardiovascular functions (2e). For the most part, only atypical or mild forms of vitamin B_1 deficiency are common in the Occident as a result of our nutritional habits (5c). In the Far East, however, the characteristic form of severe avitaminosis B_1, or beriberi, is very prevalent.

One International Unit (I.U.) of vitamin B_1 equals one U.S.P. Unit, and both are equivalent to 3 micrograms of thiamine chloride. The National Research Council (5d) recommends the following as the optimal intake of thiamine per day:

Age, years	Thiamine, mg.
Up to 1	0.4
1–3	0.6
4–6	0.8
7–9	1.0
10–12	1.2
Girls 13–15	1.4
16–20	1.2
Boys 13–15	1.6
16–20	2.0
Woman, 56 Kg., moderately active	1.5
Man, 70 Kg., moderately active	1.8

Eddy and Dalldorf (5e) thus summarize what has been learned about the metabolism of thiamine in the human body: "When thiamine is fed and taken up from the intestine it is largely converted to diphosphothiamine and stored as such in liver and kidney. As it is needed in other tissues it is dephosphorylized and travels to the tissues as free thiamine and these either rephosphorylate it (to cocarboxylase) or it is excreted in the urine as thiamine." However, food is not the only source of vitamin B_1. Najjar and Holt (27,28) have found thiamine synthesis by intestinal bacteria in adolescents, although ultimately the bacteria need an outside supply of thiamine with which to carry on their activity.

The foregoing brief summary may help the pediatrician to understand the principles, the significance, and the limitations of tests for vitamin B_1 nutrition. Of the several methods recently developed, a few are described below.

ASSAY OF 24 HOUR URINARY OUTPUT OF VITAMIN B_1

Chemical methods of urinalysis for thiamine are chiefly based on either of two reactions, and the procedures of both methods are complex and laborious. In the method outlined by Jowett (29), thiamine is oxidized to the yellow-blue fluorescent compound thiochrome, and this compound is measured in an ultraviolet fluorometer. In the methods described by Emmett, Peacock, and Brown (30), and Alexander and Levi (31), the thiamine is combined with a diazotized amino compound. The intensity of the color reaction (Ehrlich-Pauly reaction, Prebluda-McCollum reaction) is then measured in the colorimeter.

Although the daily urinary excretion of thiamine by healthy persons on an adequate diet is considerably higher than that of subjects with vitamin B_1 deficiency, no information on body stores of the vitamin is provided by the urinary excretion test, nor can one draw any conclusions as to the state of vitamin B_1 nutrition. Furthermore, dietary intake of thiamine interferes with attempts to correlate urinary excretion and body stores of the vitamin.

THIAMINE LOADING OR TOLERANCE TEST

The method described is that of Harris and Leong (32). In this test the increment in urinary excretion of the vitamin is measured after oral or parenteral administration of a test dose of thiamine. The response to this test is one of the best criteria of a subject's thiamine nutrition, and has been used as such in pediatric studies (27,33). However, the amount of thiamine which can be recovered in the twenty-four hour urine specimen after a test dose is conditioned not only by the body stores of vitamin but by other factors as well. Oral ingestion of the test dose may result in erroneous results because of impaired intestinal absorption, and parenteral administration of the test dose may lead to errors because the renal threshold for thiamine may be impaired. Furthermore, it has not yet been clearly defined what the relationship is between the amount of thiamine given in the test dose and the amount excreted in the urine (34).

FASTING HOUR EXCRETION TEST

Holt (35) has suggested examination of urine collected during the thirteenth hour after a meal, without resort to a test dose, as a means of avoiding the interference of dietary intake of the vitamin. The patient has his usual evening meal at 7 P.M. The next morning at 7 A.M. he voids and then drinks a glass of water. An hour later, at 8 A.M., a urine specimen is taken for analysis. If this urine specimen cannot be obtained exactly 1 hour after the first voiding, the time interval is noted, and the excretion is calculated on the one-hour basis. Breakfast is permitted only after the second voiding.

Absence of thiamine in the urine is a sign of thiamine deficiency.

ASSAY OF BLOOD CONTENT OF VITAMIN B_1

This is an impracticable method, since the concentration of the vitamin in the blood is too small (1 γ per 100 cc.) to be analyzed in blood samples (36).

ASSAY OF BLOOD CONTENT OF PYRUVIC ACID

The clinical value of blood pyruvic acid assays has increased since Bueding and Wortis (37) have succeeded in developing an accurate analytic method. In adults, the blood concentration of pyruvic acid normally ranges between 0.77 and 1.16 mg. per hundred cubic centimeters, with an average of 1.0 mg., and the range in children is almost identical (38). Values above 1.3 mg. are considered abnormal (39), and have been observed in illnesses associated with thiamine deficiency. As a result of the lack of thiamine pyrophosphate (cocarboxylase), pyruvic acid cannot be oxidized at the normal rate and its blood level rises (40).

BLOOD PYRUVIC ACID CURVE AFTER INGESTION OF GLUCOSE

Bueding, Stein, and Wortis (41) have suggested that the "biochemical lesion" associated with thiamine deficiency can be better shown if the patient undergoes the additional stress of metabolizing a test dose of dextrose. One hour after ingestion of 1.75 Gm. of dextrose per kilogram of body weight, adults normally show a maximum rise of 0.14 to 0.93 mg. pyruvic acid per 100 cc. of blood above the fasting level. By the third hour, the curve has declined to the fasting level. In thiamine-deficient subjects, the pyruvemia is not only greater than normal, but the maximum rise occurs later and the return to the fasting level is slower (4–5 hours) (1). However, such results are obtained only in fairly advanced states of deficiency, as demonstrated clinically by acute peripheral neuropathy or Wernicke's disease. The test is ineffective in detecting the mild deficiencies occurring most commonly in the Occident.

Summary

To summarize, the "fasting hour excretion test" provides a clear and relatively simple demonstration of thiamine deficiency. The blood pyruvic acid curve after glucose ingestion is helpful in evaluating the degree of thiamine depletion, while the thiamine loading test will uncover early states of deficiency not demonstrable by other tests. How significant the results of these tests in children are still remains to be shown, all the more so as normal values and standards in relation to age have not yet been established.

VITAMIN C

Vitamin C or ascorbic acid is present in all living plants, mainly in the actively growing parts such as leaves and flowers, and is widely distributed in animal tissues. Of the plants, citrus fruits, green vegetables, and apples contain particularly high amounts of the vitamin, while among the animal tissues those with the highest metabolic activity—pituitary body, corpus luteum, adrenal cortex, thymus gland in children, and liver (42)—have the highest vitamin C content.

Vitamin C is a hexose derivative, a monobasic sugar acid with one keto group. Its chemical name is *l*-ascorbic acid, its empiric formula is $C_6H_8O_6$. Its chemical structure puts vitamin C into the class of reductones—substances which form reversible oxidation-reduction systems. Ascorbic acid is readily converted into dehydroascorbic acid; this first oxidation step is reversible, i.e., the dehydroform can be changed back to *l*-ascorbic acid in the human body.

Vitamin C is soluble in water; in solution, it is very sensitive to atmospheric oxygen and heat, and is the most easily destroyed of all the known vitamins (43). The vitamin is available in crystalline form, which is obtained by extraction from natural sources or by synthesis.

One International Unit (I.U.) equals one U.S.P. Unit, and both are equivalent to 0.05 mg. of pure ascorbic acid.

Vitamin C is the only antiscorbutic compound which occurs naturally (2f). Both *l*-ascorbic acid and dehydroascorbic acid are biologically active, but their mode of action in the human organism is not yet well defined. Among its many functions, experimental evidence (2g) points to its role of hydrogen carrier in cellular respiration, its participation in the formation of various intracellular colloidal substances, such as cartilage, dentine, and bone material, and its role in calcium and carbohydrate metabolism.

Ingested ascorbic acid is absorbed from the intestinal tract and transported by the blood throughout the body. Both *l*-ascorbic acid and dehydroascorbic acid are present in animal tissue, but only the dehydro form is found in the blood. Any excess of the vitamin is excreted mainly by way of the urine, and only to a very small extent by way of the feces. The concentration of vitamin C in body fluids and tissues is expressed in terms of milligrams of ascorbic acid.

Since the vitamin is not synthesized in the human body, it must be supplied from the outside. Present standards set the minimum daily requirements at 30 mg. of ascorbic acid, average optimal intake varying with age. Infants need about 3 to 8 mg. per kilogram of body weight per day; children, from 5 to 7.5 mg. per day; adults, from 0.5 to 1.6 mg. per day (2h).

With regard to the many laboratory and clinical tests that have been suggested as criteria of vitamin C nutrition, one might quote the opinion of Eddy and Dalldorf (5f): "We are surfeited with tests for ascorbic acid deficiency but still hungry to know what deductions can safely be drawn from them." An extensive discussion of the metabolism of vitamin C in the adult may be found in the notable review of this subject by Ralli and Sherry (42).

Laboratory tests of vitamin C nutrition may be classified as follows: (*1*) Excretory tests, comprising (*a*) 24 hour urinary excretion of ascorbic acid; (*b*) tolerance tests, which measure urinary excretion 3, 5, or 24 hours after administration of a test dose of ascorbic acid; and (*c*) saturation tests, which measure urinary excretion in response to prolonged administration of the vitamin. (*2*) Determination of plasma content of vitamin C, comprising (*a*) concentration of ascorbic acid with the subject in a fasting state;

(*b*) vitamin C tolerance curve; and (*c*) ascorbic acid index. (*3*) Determination of vitamin C content of the white blood cell–platelet layer.

The clinical tests consist of a skin capillary fragility test and an intradermal test.

Determination of Urinary Ascorbic Acid

Most of the methods for determining the ascorbic acid content of urine are based on the reduction of an indophenol dye and the resultant color change, the so-called Tillmans' reaction (44). The degree of reduction can be measured titrimetrically or colorimetrically. While many clinical workers prefer the titrimetric methods because of their reputed relative simplicity, the colorimetric determinations are more accurate and are neither laborious nor complex. Only one of the several methods in each group will be given in detail here.

Indophenol is reduced only by ascorbic acid, not by dehydroascorbic acid. If information on the presence of both forms is desired, the dehydro form must be converted into ascorbic acid by treating the urine with hydrogen sulfide before the indophenol dye is added. The determinations for ascorbic acid may be interfered with by the presence in the urine of other reducing substances; this difficulty is taken into account in the colorimetric method.

Titrimetric Determination, According to Harris and Ray (45,46).

Reagents.

(*1*) Glacial acetic acid.

(*2*) 1 per cent starch solution

(*3*) 0.01 *N* iodine solution.

(*4*) Standard ascorbic acid solution (page 277).

(*5*) Sodium 2,6-dichlorophenol-indophenol reagent. Dissolve 50 mg. of the dye in 30 cc. of boiling water, cool, transfer to a 50 cc. volumetric flask, and dilute to volume with distilled water. If stored in a dark bottle in the refrigerator, the reagent will keep for about 3 weeks.

The indophenol reagent should be standardized against the standard ascorbic acid solution; for directions see page 278. Directions for checking the standard ascorbic acid solution against 0.01 *N* iodine will be found on page 277.

Technic. The volume of urine collected during a given period of time is measured, acidified by the addition of one-tenth its volume of glacial acetic acid, and filtered if not clear; a microburet is filled with the acidified urine. Exactly 0.05 cc. of indophenol reagent is measured with a micropipet into a centrifuge tube and urine is slowly and carefully added from the microburet until the color of the reagent is definitely blanched. If the concentration of ascorbic acid in the urine is high, so that less than 0.5 cc. is required to discharge the color of the reagent, the urine is diluted with a known volume of water and the titration is repeated. The end point is best found by matching with a control tube containing urine but no dye.

Calculation. By inserting the various values, as determined above, in the following equation, one obtains the milligrams of ascorbic acid in the urine specimen.

$$\text{Mg. ascorbic acid} = \frac{0.88 \times (\text{cc. I}_2 \Bumpeq 1 \text{ cc. standard}) \times \text{vol. urine specimen}}{(\text{cc. dye} \Bumpeq 1 \text{ cc. standard}) \times 20 \times (\text{cc. urine used in titration})}$$

where 0.88 is the amount of ascorbic acid equivalent to 1 cc. of the iodine solution, (cc. $I_2 \Bumpeq$ 1 cc. standard) is cubic centimeters of 0.01 *N* iodine solution needed in titration against 1 cc. of standard ascorbic acid solution, (cc. dye $\Bumpeq$ 1 cc. standard) is cubic centimeters of indophenol reagent needed in titration against 1 cc. of standard solution of ascorbic acid, and (cc. urine used in titration) is titration value obtained in the titration of 0.05 cc. of reagent against the urine specimen multiplied by 0.9, the correction for the amount of glacial acetic acid which has been added to the urine.

Example. 1.58 cc. of 0.01 *N* iodine solution and 2.06 cc. of indophenol reagent, respectively, were needed for the titration of 1 cc. of standard ascorbic acid solution. 1.9 cc. of urine, i.e., 1.71 cc. after correction, were used for the titration of 0.05 cc. of indophenol reagent. The volume of the urine specimen was 135 cc.

$$\frac{0.88 \times 1.58 \times 135}{2.06 \times 20 \times 1.71} = 2.64 \text{ mg. ascorbic acid in urine specimen}$$

Electrocolorimetric Determination, According to Bessey (47). *Apparatus.* Evelyn photoelectric colorimeter with filter no. 520 inserted, or any equivalent instrument.

Reagents.

(*1*) 2,6-Dichlorophenol-indophenol solution. Dissolve approximately 8 mg. of the crystals in 500 cc. warm water, cool, and filter. Store in the refrigerator when not in use. The solution keeps for about 10 days.

(*2*) 6 per cent metaphosphoric acid stock solution. Dissolve the clear sticks of glacial metaphosphoric acid in distilled water and filter; this solution keeps for 15 days if stored in the refrigerator at 5 C. Make a 3 per cent solution as required.

(*3*) Citrate buffer solution. Dissolve 21 Gm. citric acid in 200 cc. of normal carbonate-free sodium hydroxide and dilute to a volume of 250 cc.

Technic. For the determination of *preformed ascorbic acid*, 25 cc. of 6 per cent metaphosphoric acid solution are added to 25 cc. of urine, and the mixture is centrifuged or filtered if it is not clear. Then 7 cc. of the citrate buffer are added to 25 cc. of the acid urine. To make a rough estimate of its vitamin C content, an aliquot of this buffered urine is run from a 5 cc. graduated pipet into 4 cc. of the indophenol solution until the die is completely reduced. If 4 cc. of the buffered urine will reduce the 4 cc. of dye incompletely, the urine has the concentration required for the colorimetric measurement. If a smaller volume brings about reduction, an aliquot of the buffered urine is diluted with a 3 per cent metaphosphoric acid – citrate buffer mixture (25:7) to the right concentration, and this is then used for the colorimetric determination.

The photoelectric colorimeter is set so that the galvanometer reads 100 for a tube of distilled water. Into 4 cc. of the aqueous dye solution in a colorimeter tube 4 cc. of the buffered and diluted urine are gently blown from a pipet, and the tube is inserted into the machine within 5 seconds. A reading is taken 15 seconds after the initial mixing (G_{s_1}) and a second reading 15 seconds later (G_{s_2}). A small crystal of ascorbic acid is then added to the contents in the tube and a reading taken after the dye has been completely reduced (G_{s_r}). This gives a measure of the turbidity or color of the urine and is used to make a correction as follows. The machine is adjusted so that a tube containing 4 cc. of a 3 per cent metaphosphoric acid–citrate buffer mixture (25:7), plus 4 cc. of the dye and a crystal of ascorbic acid (completely reduced), reads the same as the reduced sample (G_{s_r}). With the instrument set in position, the concentration of the dye reagent (blank reading) is determined as follows—4 cc. of 3 per cent metaphosphoric acid buffered to the same *p*H as the urine are mixed with 4 cc. of the dye reagent in a tube, and read in the instrument after 15 minutes (G_b).

Calculation. The amount of ascorbic acid in the sample solution is computed with the equation

$$C = K_1 (\log G_s - \log G_b)$$

The value for K_1 must be previously determined on buffered metaphosphoric acid solutions of pure ascorbic acid as described in the directions for using the instrument (8,48). The solutions are standardized by iodine titration (page 277). Under the conditions of the test method described, and with the use of the Evelyn electrocolorimeter, the value of K has been determined as 0.088 (±0.002).

If there is an appreciable drift as a result of slower dye reduction due to substances other than ascorbic acid, the value G_s is obtained by the formula

$$G_s = G_{s_1} - (G_{s_2} - G_{s_1})$$

Dehydroascorbic acid is determined by slowly bubbling hydrogen sulfide for 15 minutes through 5–7 cc. of the buffered urine in a 15 cc. conical centrifuge tube, allowing it to stand for 2 hours at room temperature, and then freeing the hydrogen sulfide by bubbling with wet tank nitrogen for 45 minutes. Total ascorbic acid is then determined as described above for preformed ascorbic acid. The difference between the two results represents the dehydroascorbic acid.

DETERMINATION OF 24 HOUR URINARY EXCRETION OF VITAMIN C

According to Ralli and Sherry (42), the amount of ascorbic acid excreted in the urine during 24 hours is not a satisfactory criterion of vitamin C nutrition. In interpreting the results of such a determination, the potential influence of various factors, such as dietary vitamin C intake preceding the test, the amount actually absorbed, quantity of urine secreted, kidney function, and age, must be taken into account. Until the effect of these factors is clearly defined, the 24 hours excretion test remains a poor index of vitamin deficiency and nutrition.

Rally and Sherry suggest that estimation of the one hour excretion after the first morning micturition may prove to be a more reliable method. This measurement of "basic ascorburia," first proposed by Vauthey (49), would correspond in principle and significance to Holt's fasting hour excretion test for thiamine (page 264). A simplified procedure has also been suggested by Harris and Abbasy (50).

EXCRETORY VITAMIN C TOLERANCE TESTS

To increase the diagnostic significance of the 24 hour urinary excretion of vitamin C, tests have been designed to measure the increase in the urinary excretion of the vitamin in response to a test dose of ascorbic acid. These tests are based on the theory that tissues depleted of vitamin C would retain a loading dose of the vitamin more readily than those partly or totally saturated with the vitamin. In adults this method has proved to be of definite value. Oral administration of the test dose, as originally recommended by Harris and Ray (45), leads to erroneous results. It can be obviated by parenteral administration of the test dose. Collection and analysis of 24 hour urine specimens are no longer necessary, since it has been found that determinations on specimens during 3 hours after administration of the test dose yield equally good results.

PEDIATRIC CONSIDERATIONS

In small children these tests are hampered by the difficulties inherent in any method based on the analysis of urine specimens which must be collected during a given period of time. Pediatricians have therefore made little use of excretory vitamin C tolerance tests, and the plasma loading test (page 282) is commonly preferred. Nevertheless, since excretory tests form part of many reports on vitamin C nutrition, a method will be described which is equally suited for testing adults or children over the age of 5 years.

PROCEDURE

The method described is that of Ralli, Friedman, and Kaslow (51).

The bladder is emptied in the morning and urine is collected for the 3 hours thereafter (specimen 1). The following morning, after the bladder has been emptied, 100 mg. of ascorbic acid in about 5 cc. of physiologic saline solution are injected intravenously. Urine is again collected for 3 hours (specimen 2). Both specimens are analyzed for vitamin C content by one of the methods described (page 267). If the Harris-Ray method is used, glacial acetic acid, 10 per cent by volume, may be added to each specimen as a preservative, and determination delayed for 24 hours. The addition of the acid simultaneously accomplishes the acidification of the urine, which is the first analytic step of this method. If glacial acetic acid is not added, the determination is carried out by the Bessey method, immediately after the specimens are obtained.

INTERPRETATION

Normally, if the diet of the subject tested contains adequate amounts of vitamin C, approximately 50 per cent of the test dose is excreted within 3 hours. Subjects whose diets are low in vitamin C show greater avidity for vitamin C, excreting only about 15 per cent of the test dose within 3 hours, while scorbutic patients excrete less than 5 per cent within the same period.

TABLE 45

Results of Excretory Vitamin C Tolerance Test in Children with and without Clinical Avitaminosis C

Age, months	Condition	Ascorbic acid, mg. administered	Ascorbic acid, mg. excreted in 24 hrs. after ingestion of test dose
7	Scurvy	100	0.7
8	Scurvy	85	<0.4
8	Scurvy	170	<0.5
11	Subscurvy	100	<0.3
9	Scurvy cured	100	10.9
13	Normal	100	5.0
38	Normal	100	8.0

From Harris and Ray (45).

Patients failing to excrete substantial amounts of the test dose while they are in a state of vitamin deficiency begin to excrete increasing amounts as they become saturated with the vitamin as a result of dietary treatment (Fig. 35). Table 45 illustrates the strikingly different excretory response of scorbutic infants and of infants recovering from the disease. The infants were given the loading dose by mouth and the 24 hour urinary excretion was measured (page 270).

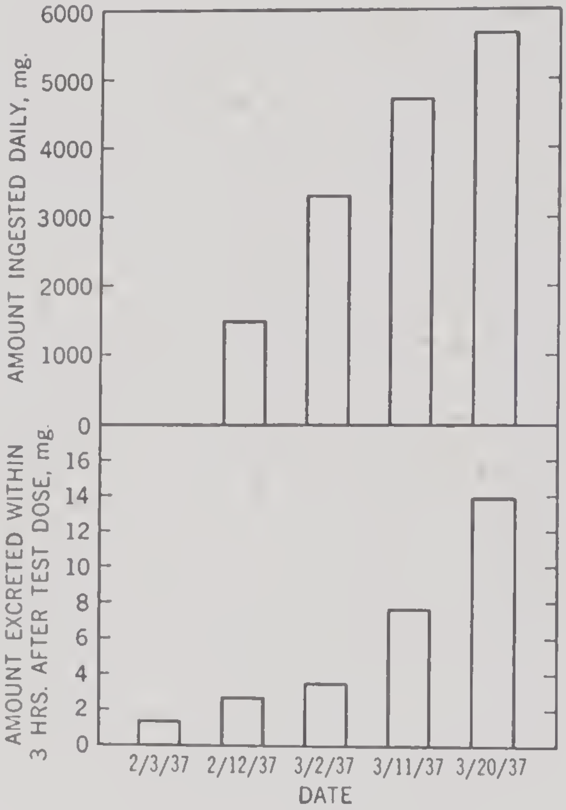


Fig. 35. Intravenous vitamin C tolerance tests with 100 mg. ascorbic acid in a case of scurvy. Three hour excretory response in relation to daily intake of vitamin C. From Ralli and Sherry (42).

VITAMIN C SATURATION TESTS

Such tests measure the response of the organism to daily oral administration of ascorbic acid. Until the subject's tissues have become saturated with the vitamin, the largest part of the test doses are utilized, so that only small amounts are excreted in the urine. As the tissues approach a state of saturation, increasingly larger amounts of the ingested vitamin are excreted in the urine. The excretion of a definite amount of the vitamin marks the saturation point. The time, in days, that it takes to reach the saturation level is taken as the measure of the subject's state of vitamin C saturation. The smaller the previous intake, the longer is the period required to reach saturation.

PEDIATRIC CONSIDERATIONS

Because of technical difficulties, the test has been used relatively little in children. The original procedure calls for collection and analysis of 24 hour urine specimens. Subsequent investigation revealed that 80 per cent of the total 24 hour increase in excretion occurred within 5 to 6 hours after administration of the test dose, and the procedure was made considerably simpler. Harris (52) has recently recommended that a daily urine specimen be collected only during the 2 hours that the urinary excretion of the vitamin is at its peak. Such a modified procedure is much easier to apply to children, and it has even been possible to carry out mass surveys of vitamin C nutrition.

However, in infants and young children it is difficult to obtain even such a specimen at the proper time except by repeated catheterization or by placing the child on a frame. Since neither way can be recommended for routine clinical use, the plasma vitamin C loading test largely replaces the saturation test in this age group. Another reason for this substitution is that evaluation of the results of the saturation test in children below school age is far from exact, because of lack or normal standard values. Although the results obtained in scurvy are easily distinguished from those in nonscorbutic infants, there are as yet no criteria for a quantitative evaluation of test results.

PROCEDURES

The method described is that of Harris (52). The subject's weight is noted. The saturation dose of 11 mg. of ascorbic acid per kilogram of body weight, dissolved in sugar water or sweetened tea, is given by mouth at 10:30 A.M., the minimal dose being 100 mg. At 2:00 P.M. the bladder is emptied, and the urine is discarded. All the urine voided between 2:00 and 4:15 P.M. is collected and pooled, its volume is measured, and the concentration of ascorbic acid is estimated according to one of the methods described above. The same procedure is followed on each of the following days.

The test may be discontinued as soon as the tentative standard level of excretion (0.8 mg. per Kg. body weight within 2¼ hrs.) is reached, or it may be continued for an additional day or two, until the excretion no longer shows any appreciable increase.

INTERPRETATION

The number of days it takes for the steadily rising curve of the daily vitamin C excretion to reach a level of 0.8 mg. per kilogram of body weight in the 2¼ hour specimen is the criterion of the subject's state of vitamin C nutrition. In children over 5 years of age the state of nutrition is considered adequate if the level is reached by the second day of the test. Failure to reach this level within 2 days is considered a below standard response, and each subsequent day is counted as a further degree of dropping below standard. In scurvy, it takes 7 to 10 days before the urinary excretion signals the approach of saturation. Judged by these criteria, a considerable number of apparently normal children are in a state of suboptimal vitamin C nutrition. Harris (52) emphasizes that this does not imply obvious ill health. He says: "The test discloses whether the intake of vitamin C has been above or below some accepted standard of intake, and if below, how much below." A daily intake of 30 mg. of ascorbic acid (the League of Nations standard) is taken as the standard.

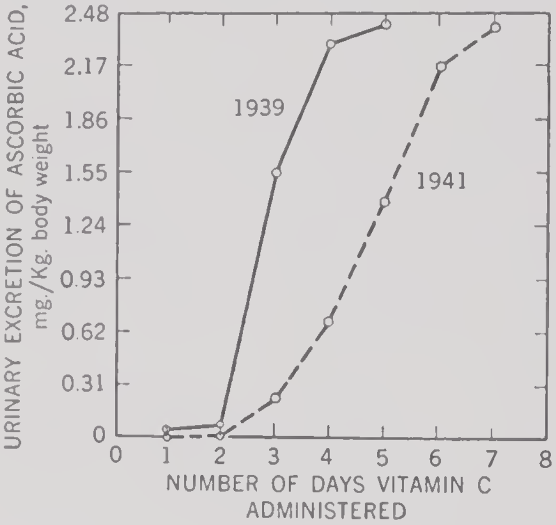


Fig. 36. Comparative results of vitamin C saturation tests in a group of boys in a peace year (1939) and in a war year (1941). From Harris (54).

The results of a mass survey of school children by means of the saturation test, illustrated in Figure 36, may serve as an example. They disclose a definite decline of vitamin C nutrition during the first years of World War II.

There is also some evidence (52) that during acute infectious diseases the response to the test is delayed, as a consequence of fever, and may simulate a low state of saturation.

There are few observations on infants and young children. Harris (52) claims that the test should be of sufficient accuracy in infants and children, as well as in adults, when allowance is made for their different needs by adjusting the recommended dosage in proportion to body weight. However, that some unforeseen fallicies may arise in the interpretation of results obtained from young children is clear from Laurin's studies on healthy breast-fed and bottle-fed infants (53). The infants were given 50 mg. of ascorbic acid at 7 A.M. and at 7 P.M. each day during the test period. Four samples of urine were collected daily between 8:30 A.M. and 7 P.M. The breast-fed infants excreted high amounts of ascorbic acid as early as the first or second day of the test, whereas in the bottle-fed infants the rise in ascorbic acid excretion was consistently delayed for many days, in many instances for as long as 12 days.

DETERMINATION OF ASCORBIC ACID IN PLASMA

Most of the methods are based on the quantitative reduction by the plasma ascorbic acid of substances which change their color when being reduced. The reagents successfully used are dichlorophenol-indophenol, methylene blue, and dinitrophenylhydrazine. Since fresh plasma contains practically no vitamin C in its hydro form, i.e., already oxidized, the results permit a fairly accurate estimate of the total vitamin C content of plasma.

The amount of reagent consumed in the oxidation of ascorbic acid can be determined by titrimetric or colorimetric determination. A number of methods have been designed for clinical use, among them the following:

(*1*) Macromethods, using 2 cc. of plasma (5 cc. of whole blood) and indiphenol: (*a*) Farmer and Abt (55) and (*b*) Ingals (56), both by titrimetric determination; (*c*) Mindlin and Butler (57) by electrocolorimetric determination.

(*2*) Micromethods: (*a*) Farmer and Abt (58), titrimetric determination, and (*b*) Mindlin and Butler (57), by colorimetric determination, both methods using 0.1 cc. of plasma (0.3 cc. of capillary blood) and indophenol; (*c*) Butler, Cushman, and MacLachlan (59), by electrocolorimetric determination, using 0.5 cc. of plasma (2 cc. of oxalated blood) and methylene blue; (*d*) Lowry, Lopez, and Bessey (60), by spectrophotometric determination, using 10 c.mm. of plasma (0.1 cc. of capillary blood) and dinitrophenylhydrazine.

PEDIATRIC CONSIDERATIONS

If blood can be obtained by venipuncture, one of the macromethods requiring 4–5 cc. of whole blood may be used to determine vitamin C content of plasma in children. But for young children, from whom such quantities are sometimes hard to get, micromethods requiring 0.1–2 cc. of whole blood, and most of which can be carried out on 0.1 cc. or less of plasma, are more convenient and at times indispensable. Photoelectric determination is preferable to titration methods; it eliminates subjective reading of end points and the need for standardizing dye solutions with each analysis. Wider use of some of the micromethods may be limited by the need for special apparatus, such as the spectrophotometer or special glassware (Farmer-Abt method), and by the fact that the procedures are laborious and require considerable skill and experience. The two methods described below were arbitrarily chosen, but they are a good compromise between the demands for high reliability and practical requirements. Both methods use metaphosphoric acid instead of tungstic acid (61) for the deproteinization of the plasma.

PROCEDURES

Macrotitration Method, According to Ingals (56).

Reagents.

(*1*) Glacial acetic acid.

(*2*) 10 per cent solution of metaphosphoric acid. Make up fresh each week and keep in refrigerator.

(*3*) 1 per cent starch solution.

(*4*) 0.01 *N* iodine solution.

(*5*) Standard ascorbic acid solution. Dissolve 100 mg. pure ascorbic acid in 100 cc. boiled distilled water. Keep the solution in a brown, glass-stoppered bottle in the refrigerator.

To check the solution, transfer 1 cc. of the standard solution to a centrifuge tube, add 1 drop of the starch solution, and titrate with 0.01 *N* iodine solution from a microburet until a permanent blue color develops. Since 1 cc. of the iodine solution is equivalent to 0.88 mg. of ascorbic acid, the strength of the standard ascorbic acid solution can be calculated easily.

(*6*) Sodium 2,6-dichlorophenol-indophenol reagent. Dissolve 20 mg. of the dye in 75 cc. boiling water, cool, transfer to a 100 cc.

volumetric flask, and dilute to volume with distilled water. This reagent will keep 3 weeks if stored in a dark bottle in the refrigerator.

To standardize the reagent, add 1 drop of glacial acetic acid to 0.2 cc. of the standard ascorbic acid solution measured into a centrifuge tube with a microburet. Titrate the mixture with the indophenol reagent from a 5 cc. microburet until a definitely pink color develops. The titration value gives the exact amount of indophenol reagent that is equivalent to 0.2 cc. of standard ascorbic acid solution.

Technic. Into a centrifuge tube containing 1 cc. of 0.7 per cent solution of lithium oxalate, evaporated to dryness, are transferred 4 to 5 cc. of blood.* The tube is centrifuged; then 2 cc. of the supernatant plasma are transferred into a test tube, 2 cc. water and 6 cc. metaphosphoric acid are added, and the mixture is stirred with a glass rod and filtered. A 5 cc. aliquot of the filtrate, equivalent to 1 cc. of plasma, is transferred into a 15 cc. centrifuge tube and titrated with the standardized indophenol reagent from a 5 cc. microburet graduated in 0.01 cc. The titration must be done rapidly. The end point is reached when a faint pink color remains for at least 25 seconds. A blank, consisting of 4 cc. each of water and metaphosphoric acid, is titrated in the same manner.

Calculation. To calculate and express the results in terms of milligrams of ascorbic acid per 100 cc. of plasma, the various titration values are substituted in the equation:

$$\frac{0.88 \times (\text{cc. I}_2 \Bumpeq 1 \text{ cc. standard})}{5 \times (\text{cc. dye} \Bumpeq 0.2 \text{ cc. standard})} \times 100 \times \text{cc. dye used for titration} = \text{mg. ascorbic acid per 100 cc. plasma}$$

where (cc. $I_2 \Bumpeq$ 1 cc. standard) = cc. of 0.01 *N* iodine solution needed in titration against 1 cc. of standard ascorbic acid solution; (cc. dye $\Bumpeq$ 0.22 cc. standard) = cc. of indophenol reagent needed in titration against 0.2 cc. of standard ascorbic acid solution; and cc. dye used for titration = titration value obtained in the titration of the analyzed plasma with indophenol reagent.

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* It has been claimed that the use of potassium cyanide in addition to the oxalate prevents oxidation of the ascorbic acid in the withdrawn blood, so that blood samples can be kept for several hours in the refrigerator. However, objections have been raised to the use of cyanides, and it therefore seems advisable to omit the potassium cyanide and to analyze the oxalated blood immediately.

The formula is valid only under the test conditions as described above.

Example. For the titration of 1 cc. of standard ascorbic acid solution, 1.56 cc. of iodine solution were used. For the titration of 0.2 cc. of standard ascorbic acid solution against indophenol reagent, 2.05 of the reagent were needed. For the titration of the deproteinized plasma, 0.08 cc. of indophenol reagent was needed (after deduction of titration value for the blank). Therefore:

$$\frac{0.88 \times 1.56}{5 \times 2.05} \times 100 \times 0.08 = 1.07 \text{ mg. ascorbic acid per 100 cc. plasma}$$

Electrocolorimetric Micromethod, According to Mindlin and Butler (57).

Apparatus.

(*1*) Photoelectric colorimeter, for instance, the microcolorimeter of Evelyn and Cipriani (8) which was used in developing the method. Use Evelyn filter no. 520, which transmits 494–562 mμ. If this filter is too opaque, filter no. 520M, which transmits 456–610 mμ may be used. The colorimeter cell is equipped with a glass plunger, the height of which is so adjusted that it will be just below the surface when the cell contains 1.1 cc.

(*2*) Micropipet, calibrated to contain 0.1 cc. and to deliver 0.15 cc. The authors recommend the modified Shohl tip micropipet. A glass Luer adapter is sealed to the bottom end of the micropipet, the ground end fitting snugly into a hypodermic needle of small gage (18–23) which is cut off horizontally and ground on a stone.

(*3*) Davies tubes. These are glass tubes, 8 cm. long and with an internal diameter of 3–4 mm., capped tightly with rubber at each end.

Reagents.

(*1*) 20 per cent potassium oxalate solution.

(*2*) 5 per cent and 2.5 per cent metaphosphoric acid solutions. Make up fresh at least every 2 weeks, and store in the refrigerator.

(*3*) 2,6-Dichlorophenol-indophenol solution. Dissolve 1.2 mg. of the dye in 100 cc. distilled water at 85–95 C., filter, cool, and dilute so that the solution gives a galvanometer reading of 80–85 when distilled water reads 100. Store in a dark bottle in the refrigerator. The solution keeps for 3 weeks.

(*4*) Sodium acetate solution. Make up 1.51 Gm. of $NaC_2H_3O_2$-

$3H_2O$ to 100 cc. with distilled water and adjust to pH 7.0 with 0.11 cc. of 0.5 M acetic acid. If standardized acetic acid is not available, adjust the pH of this solution by adding dilute acetic acid, a few drops at a time, and testing small aliquots with bromothymol blue. Add a few drops of toluene to the solution to ensure its clarity on standing.

(*5*) Indophenol–acetate solution. Equal volumes reagents *3* and *4*.

A number of Davies tubes are filled with oxalate solution, drained quickly, and dried in the oven for 15 minutes at 105 C.

Into a Davies tube thus prepared about 0.3 cc. of capillary blood from a pricked finger or heel is collected, the tube is closed with the rubber caps at both ends, and centrifuged. The cap above the plasma is then removed; 0.1 cc. of plasma is carefully drawn into the special micropipet and transferred to a 15 cc. conical centrifuge tube. With the same pipet 0.15 cc. distilled water is added, thus washing out the pipet; then exactly 0.2 cc. of the 5 per cent metaphosphoric acid is added, the mixture is stirred, and centrifuged for at least 5 minutes or until the supernatant fluid is clear.

The galvanometer is set at the center setting; this is obtained when the dye solution, upon complete reduction by an excess of ascorbic acid, reads 100. If this setting is once known, the determination need not be made again as long as the setting for distilled water with the proper filter and tube remains the same. This is checked at each analysis.

The center setting of the galvanometer being adjusted, 0.8 cc. of indophenol–acetate solution is measured into the microcell of the colorimeter. With an accurately calibrated, fine-tipped pipet, 0.3 cc. of the supernatant fluid is removed from the centrifuge tube without contamination by the precipitate and added to the indophenol–acetate solution in the microcell. The mixture is stirred, the plunger is placed in position, and the cell is inserted into the colorimeter. Readings are taken 30 seconds after addition of the supernatant fluid, and 1 and 2 minutes later, to make sure that there has been no significant increase in reduction due to substances other than ascorbic acid.

For the blank, 0.8 cc. of the indophenol–acetate solution is measured into the microcell, 0.3 cc. of the 2.5 per cent metaphosphoric acid is added, the mixture is stirred, the cell is inserted into the colorimeter, and the reading noted immediately.

Calculation. The concentration of ascorbic acid in a sample solution is calculated by the equation

$$C = K(\log G_s - \log G_b)$$

where G_s is the galvanometer reading for the sample solution and G_b the galvanometer reading of the blank.

The value of K depends upon the dye solution and the type of photoelectric colorimeter used; it is established by a few determinations on carefully measured samples of varying, known concentrations of crystalline ascorbic acid dissolved in 2.5 per cent metaphosphoric acid. Butler and Mindlin found the value of K, for the method and apparatus used by them, to be 0.028 with filter no. 520M, and 0.023 with filter no. 520. Concentration of ascorbic acid in the sample may be converted to milligrams per 100 cc. of plasma by multiplying C by 1,500.

INTERPRETATION

Table 46 shows Ingals' evaluation of ascorbic acid levels in plasma.

TABLE 46
Evaluation of Ascorbic Acid Levels in Plasma

Fasting level of ascorbic acid, mg./100 cc.	State of vitamin C nutrition	Clinical condition indicated
1.0 –2.0	Saturation	—
0.7 –1.0	Normal	Normal health
0.5 –0.7	Low normal	Normal health
0.3 –0.5	Suboptimum	Normal health, temporarily limited intake
0.15–0.3	Deficiency	Almost diagnostic of asymptomatic scurvy
0–0.15	Deficiency	Almost diagnostic of scurvy

Adapted from Ingals (56).

The fasting values in an average healthy child on an adequate intake are about 0.7 to 0.9 mg. of ascorbic acid per hundred cubic centimeters. Plasma levels respond very promptly to a change in the vitamin C intake, and these rapid fluctuations render reliance upon a single determination inadvisable. Subnormal and even low values may be the result of sporadic low intake; they do not necessarily indicate that any marked reduction of the tissue nutrition has taken place. To eliminate this source of error, both the vitamin C loading test and the saturation test will be found of value.

PLASMA VITAMIN C LOADING TEST

The test is designed to probe a subject's ability to dispose of a single, massive dose of ascorbic acid. This is accomplished not by measuring the urinary excretion of ascorbic acid, as in the excretory test described on page 271, but by following the changes that take place in the vitamin C level in the plasma.

Oral administration of 100 mg. or more of ascorbic acid produces a significant rise in the plasma level within 1 hour, followed by a gradual fall to the original level within 4 to 6 hours (62,63). In subjects with inadequate vitamin C intake the curve is flatter, and the return to the starting level is more rapid.

When the test dose is injected intravenously (64), thus eliminating the factor of absorption, the peak is reached within 3 to 6 minutes after injection, there is a rapid fall during the first 1½ hours, and a gradual approach to the original level in 3 to 4 hours (Fig. 37). Here again the maximum rise shows some relationship to the preceding dietary intake (65).

Evaluation of the results of the intravenous loading test is based on the rate at which the injected ascorbic acid disappears from the blood, as shown by the plasma curve covering only the first hour after injection. Recently, however, Kajdi, *et al.* (66) have suggested that the last phase of the curve be used as a criterion, believing that the difference between the vitamin level in the plasma immediately before injection of the loading dose and the level 4 hours later provides a reliable index of vitamin C depletion.

PEDIATRIC CONSIDERATIONS

The disadvantages of the excretory loading test as a pediatric procedure are obvious (page 271), although its usefulness is unquestionable. The more practicable method for testing children is the plasma loading test, particularly in the modified form as described below, which calls for only 2 blood samples.

PROCEDURE

According to the method of Kajdi, Light, and Kajdi (66), the first blood sample is obtained in the morning, with the child in the fasting state. Immediately thereafter, 200 mg. of ascorbic acid, dissolved in 5 to 8 cc. of saline, are injected intramuscularly. The

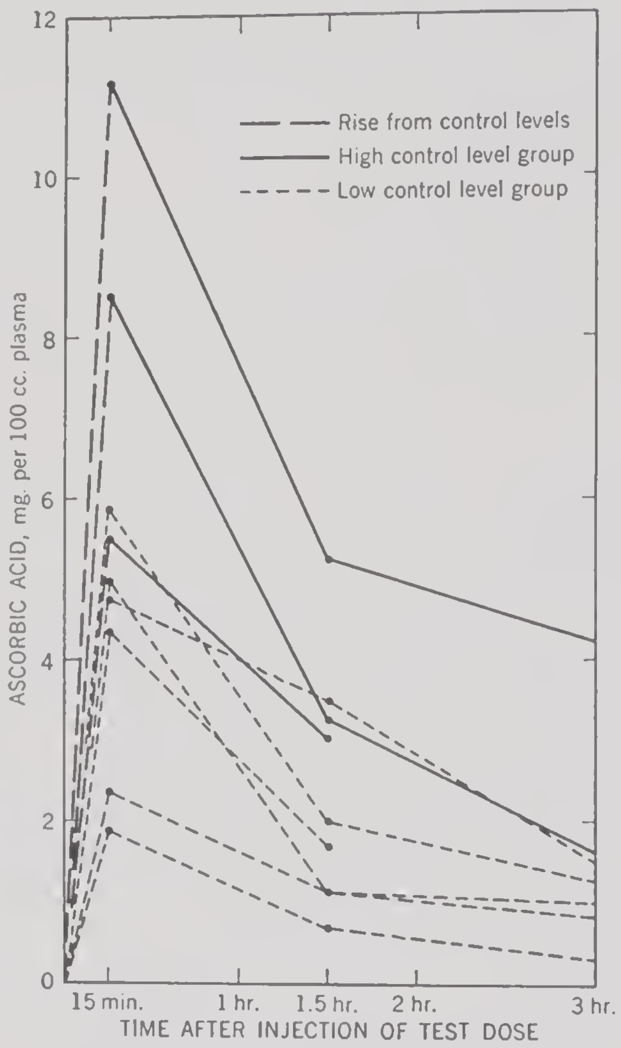

Fig. 37. Typical plasma vitamin C loading curves in adults after intravenous injection of 1 Gm. of ascorbic acid. From Wright, Lilienfeld, and McLenathen (65).

second blood sample is taken 4 hours later. Both specimens are analyzed for vitamin C content immediately after they are obtained, by one of the methods already described (pages 277–281).

INTERPRETATION

The state of vitamin C nutrition is reflected by three different values: (*1*) Fasting level of ascorbic acid in plasma (page 281). (*2*) "Final concentration" of ascorbic acid in plasma, i.e., the level

4 hours after injection of the loading dose. In normal children, the final concentration is greater than 0.6 mg. per hundred cubic centimeters; in children with moderate to serious deficiency, the levels are 0.2 to 0.6 mg.; in scurvy, the levels are below 0.2 mg. (*3*) The vitamin C index, obtained by multiplying by 100 the product of the fasting level and the difference between fasting level and final concentration.

Example. Fasting value, 0.8; final concentration, 0.9; difference, 0.1.

$$\text{Index} = 0.8 \times 0.1 \times 100 = 8$$

Normally, the index of children with an adequate vitamin C intake is greater than 6, and usually is above 10. Indices below 6 indicate deficiency states, and the lower the index the more serious is the tissue depletion. In active scurvy indices are below 0.9.

VITAMIN C CONTENT OF THE WHITE CELL–PLATELET LAYER OF BLOOD

An observation by Butler and Cushman (67) that ascorbic acid is still to be found in the blood cells when the plasma shows a state of depletion, led them to realize that the vitamin C content in the white cell–platelet layer is the last to go, and that it may therefore be found in normal quantities when the plasma level is already reduced to "scorbutic" values. According to Butler (68) "the vitamin C concentration of the white blood cells and platelets provides an index of vitamin C deficiency that extends beyond the limits defined by plasma values."

As determined by the method of Butler, Cushman, and MacLachlan (59), the normal vitamin C concentration in the white cell–platelet layer varies between 25 and 38 mg. of ascorbic acid per 100 Gm. of white blood cells. The method requires approximately 15 cc. of whole blood. According to Ralli and Sherry (42), the method is "both tedious and difficult and will probably not be adaptable for routine laboratory use."

CAPILLARY FRAGILITY SKIN TEST

The weakening of the endothelial wall of the capillaries is a characteristic pathologic change in scurvy, and it was customary, not so long ago, to test capillary resistance as a measure of the degree of vitamin C deficiency. Development of more exact methods has caused the fragility test to be considered an insufficiently reliable assay of vitamin C nutrition, except in scurvy (69,70). For a description of the various methods, see page 310.

INTRADERMAL TEST FOR VITAMIN C DEFICIENCY

Rotter's test (71) is based on the observation that a dye solution of trichlorophenol-indophenol becomes decolorized when injected intradermally. The time required for complete decolorization is considered an index of vitamin C saturation of the tissues. The test, however, is not specific, since other reducing substances may also be involved in the bleaching process (72,73). Nevertheless, a description of the test seems warranted, in view of assertions (74) that the test is of value.

PROCEDURE

The method described is that of Portnoy and Wilkinson (75).

Reagent. Sterilize a solution of 2 mg. indophenol in 4.9 cc. water by autoclaving, or pass 4 mg. indophenol dissolved in 4.9 cc. water through a Seitz filter. As both solutions have a higher concentration than desired, dilute to the correct strength (2 mg. dye in 4.9 cc. water) by removing an aliquot with a sterile pipet and titrating against a standardized solution of ascorbic acid (page 277). Then add sufficient sterile water to the measured bulk of the solution to obtain the desired concentration.

Technic. The skin of the forearm is cleansed with ether or alcohol and permitted to dry. Exactly 0.01 cc. of the dye solution is injected under the epithelium so that a wheal about 2 mm. in diameter is raised. The length of time required for the color of the dye to disappear completely is noted with a stop watch. Three more wheals are raised, and the average time for decolorization is calculated.

INTERPRETATION

Decolorization times of 10 minutes or less are regarded as normal; 10 to 14 minutes are considered indicative of borderline states; and 14 minutes or more are a definite sign of Vitamin C deficiency.

VITAMIN D

Vitamins D are present only in the animal and human organism, with the greatest amount in fish livers. Provitamins D, which are members of the sterol family and occur in plants as well as in the animal organism, are converted into the vitamins D by ultraviolet and cathode ray irradiation. According to Rosenberg (2i) provitamins D "differ from each other only in the number of carbon atoms in the side chain and the degree of unsaturation. The sterol skeleton is the same for all provitamins. This is also true for the vitamins D derived from the provitamins D. All chemically investigated vitamins D have the same constitution with the exception of different structures of the side chain." Table 47 gives the relationship between the five known vitamins D which have been prepared in pure form, and their provitamins.

TABLE 47
Vitamins D and Their Provitamins

Vitamin D*	Corresponding provitamin
D_1: a molecular compound of vitamin D_2 and lumisterol$_2$..	Ergosterol
D_2: also called caliciferol and viosterol; derived from plant sources..........................	Ergosterol
D_3: also called dimethyldihydrocalciferol; chief form of various vitamins D found in cod liver oil; formed in skin and milk through irridation......	7-Dehydrocholesterol
D_4..	22-Dihydroergosterol
D_5..	7-Dehydrositosterol

Adapted from Rosenberg (2k).
* D_2–D_5 have been prepared in pure form.

Vitamins D are obtained by extraction from natural sources (fish livers) or by irradiation of the provitamins. The vitamins are soluble in fat and in the various organic solvents. None of the provitamins or vitamins D have been obtained by total synthesis.

The accepted unit of measurement of vitamins D is the International Unit, defined as 0.025 microgram of pure crystalline vitamin D_2 dissolved in 1 mg. of olive oil. One International Unit equals 1 U.S.P. Unit.

The human organism is unable to synthesize vitamin D in the quantities needed to maintain health. Under natural conditions, higher animals receive their supply of vitamin D from the dietary intake and from the irradiation products which accumulate in the skin exposed to sunlight. The daily required intake is estimated at 400 to 800 I.U. for infants, but for premature infants and twins the intake must be increased to 5,000–10,000 I.U. (76). Children over 1 year old and adolescents need an optimum of about 400 I.U.

Vitamin D is the antirachitic principle; it guarantees normal ossification of bone and stimulates growth. In its metabolic action it participates primarily in the regulation of mineral metabolism; it is also concerned with certain phases of sugar metabolism. It seems probable that there is an interrelation between this activity and endocrine and enzymic functions (5g).

So far no direct methods for assay of vitamin D have been developed, although in daily clinical work exact knowledge of the status of vitamin D nutrition is needed far more than that of any other vitamin. The indirect methods are the clinical tests for vitamin D deficiency, identical with those used to diagnose rickets. They include roentgenographic examination of the bones and determinations of inorganic phosphorus and alkaline phosphatase activity in serum. The phosphatemic curve (page 211) should also be mentioned here.

Biologic tests, particularly the rat assay method, are the only means of assessing accurately the vitamin D content of human tissues and body fluids. Warkany (77), using this method, found that in normal subjects between the ages of 7 and 39 years the vitamin D content of serum ranged between 45.9 and 135 U.S.P. Units per hundred cubic centimeters, with an average value of 99.09. It seems doubtful (78) whether the antirachitic activity of the blood, as measured by rat assay, always parallels the degree of vitamin D deficiency, as judged by clinical criteria.

VITAMIN K

This vitamin is found mainly in plants, such as alfalfa, spinach, cabbage, and cauliflower, and in microorganisms (bacteria). All parts of the plant containing chlorophyll are sources of vitamin K_1, whereas bacteria are rich in vitamin K_2. Only small amounts of vitamin K are present in the mammalian organism.

According to Dam (79), its discoverer, vitamin K may be characterized as a naphthoquinone compound. The difference between the two types of vitamin K in its natural form—K_1 and K_2—are in the side chains attached to the naphthoquinone nucleus. A highly potent synthetic compound, commercially available as menadione, is 2-methylnaphthoquinone.

The natural vitamins (K_1 and K_2) and 2-methylnaphthoquinone are fat soluble; a few water-soluble synthetic compounds are also available now.

Vitamin K is the "clotting vitamin." Its presence is essential for the formation of prothrombin and the maintenance of a normal prothrombin level in the blood. Deficiency of the vitamin leads to hypoprothrombinemia and hemorrhagic disease.

Man obtains vitamin K in two ways: in the food and by synthesis by intestinal bacteria. Provided intestinal fat absorption is normal, the fat-soluble vitamin K is absorbed by the blood, transported to the liver, and there rapidly metabolized. So far as known, no organ in man is a depot for the vitamin, and only very small amounts are found in the blood. What happens to the vitamin while it is being metabolized is still obscure. While relatively large amounts are contained in the feces, there is no urinary excretion of the vitamin.

According to Dam (79), "the unit for vitamin K activity can be based merely on the quantitative effect on the blood coagulation under fixed circumstances or on a comparison with the standard substance." One microgram of pure 2-methyl-1,4-naphthoquinone has been suggested as such a standard by the League of Nations. The equivalents (79), in micrograms (γ) of 2-methyl-1,4-naphthoquinone (MN), of some of the other units which are frequently employed in clinical studies are:

1 Dam-Glavind unit is equivalent to 0.04 γ MN
1 Almquist-Klose unit is " " 4.2 " "
1 Ansbacher unit is " " 0.5 " "
1 Thayer unit is " " 0.95 " "
1 Schoenheider unit is equal to 1 Dam-Glavind unit

Methods for direct determination of vitamin K in tissues and body fluids are so far lacking, but indirect methods permit a fair estimate of a subject's vitamin K nutrition. Clinical tests of vitamin K nutrition measure the clotting power of blood as a function of prothrombin concentration. For test procedures, see pages 49–54.

Dam (79) states that low prothrombin levels, indicating deficiency of vitamin K and increased bleeding tendency, are encountered in association with the following clinical conditions: (*1*) Simple alimentary avitaminosis K. This is very rare, since "there is ample supply of vitamin K from putrefaction processes in the intestine."

(*2*) Cholemic bleeding tendency. In obstructive jaundice and with complete bile fistula, the absorption of vitamin K is impaired because of the absence of bile from the intestine. (*3*) Hemorrhagic diathesis associated with intestinal diseases. Anatomic changes in the intestinal mucosa or hypermotility of the intestinal tract cause insufficient absorption of fat and fat-soluble vitamins in such conditions as sprue, celiac syndrome, and ulcerative colitis (page 258). (*4*) Hypoprothrombinemia of the newborn. Congenital vitamin K deficiency is the most important etiologic factor in hemorrhagic disease of the newborn (page 52). (*5*) Hypoprothrombinemia due to liver disease. Liver injury impairs the formation of prothrombin, even when there is an ample supply of vitamin K. Hypoprothrombinemia which remains unaffected by vitamin K administration indicates the presence of hepatic disease. The response of the prothrombin level of the blood to vitamin K treatment as a test of hepatic function is described on page 53.

For tests for the diagnosis of hemorrhagic conditions not related to vitamin K deficiency, the reader is referred to the methods outlined on pages 477–480.

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CHAPTER IX

Cardiovascular System

CIRCULATORY FUNCTION TESTS

The scheme currently used for a functional classification of heart disease (Table 48) clearly shows in which cases function tests are most useful. When there is clinical evidence of heart failure (Classes III and IV), function tests are not only unnecessary but may even cause additional damage to the heart (1). But in patients of classes I and II fuller information as to the reserve power of their cardiovascular system is desirable, as, for example: Are they likely to suffer from latent decompensation? How much physical activity will they tolerate?

Since the growing organism of the child withstands cardiac and circulatory strain with greater ease and far longer than that of the adult, function tests are particularly valuable for discovering masked circulatory failure in children. One group of tests, which includes chemical and gas analytic blood examination, spirometry, and determinations of circulation time, cardiac output, and venous pressure, are simply a means for revealing abnormalities due to circulatory insufficiency. However, most of these methods are impracticable for routine use, particularly in children. And while the electrocardiogram reveals and localizes the heart's action, it does not show the extent of functional impairment.

The "tolerance tests," therefore, are the most useful tests for disclosing functional impairment. The rationale of these tests is that any kind of exertion may produce reactions in individuals suffering from latent circulatory or cardiac decompensation which differ from those occurring in healthy subjects. Thus the electrocardiographic response to digitalis and to anoxemia has been used as a criterion of cardiac tolerance. Mannheimer (3) has recently modified the hypoxemia tolerance test, as employed by Levy and co-workers (4) for diagnosing coronary insufficiency in adults, to serve as a test for cardiac function in children. Confirmatory studies are not yet available.

The problem of testing circulatory function is commonly approached by attempts to correlate the response to a standard exercise with the functional efficiency of the circulatory mechanisms.

TABLE 48
Functional Classification of Heart Disease

Class	Description
I	Patients with a cardiac disorder, without limitation of physical activity; ordinary physical activity causes no discomfort
II	Patients with a cardiac disorder, with slight to moderate limitation of physical activity; ordinary physical activity causes discomfort
III	Patients with a cardiac disorder, with moderate to great limitation of physical activity; less than ordinary physical activity causes discomfort
IV	Patients with a cardiac disorder unable to carry on any physical activity without discomfort

From New York Tuberculosis and Health Association (2).

The following data have been used as criteria of a subject's response in exercise tolerance tests: (*1*) "Excess metabolism," as determined from the amount of oxygen consumed during the work and recovery periods, minus the amount used during a corresponding period before the exercise (5). This test is a valuable aid in estimating cardiac function (6). (*2*) Pulse rate and blood pressure. The effect of tolerance tests on the two gives information on total circulatory efficiency, which depends on heart function as well as on vascular tonus and nervous stability. It is a practical and simple means of measuring circulatory and myocardial function (page 306). (*3*) Electrocardiographic changes. The electrocardiographic response to tolerance tests is the only method which reveals the functional response of the heart alone; it is simple enough to be employed in children (page 307).

A great deal has been written about the usefulness, as well as the limitations, of such tests. Since circulatory capacity is the result of three functions—cardiac action, nervous regulation, and vascular tone—it is obvious that any test based upon the response to physical strain necessarily reveals the combined reactions of all three functions. Fractional analysis of the circulatory response cannot, as yet, be made. No claim can be made, therefore, that the results of any of the methods mentioned above are indicative of one functional factor only, for instance, of heart function. This is clearly shown by the observation that even such apparently pathognomonic changes as appear in the electrocardiogram after exercise also occur in noncardiac patients suffering not from heart disease but from neurocirculatory asthenia, the so-called "effort syndrome" (7). Such patients cannot be classed as patients with latent heart failure, but the fact remains that they tire on effort more easily than does the average normal person, and that this fatigue involves the function of the myocardium. Whether the one or the other mechanism is more or less involved does not detract from the great value and practical conclusions that may be drawn from the results of tolerance tests.

In Levine's (7) words, ". . . functional tests in current use are for the most part tests of physical fitness and not of cardiac disease." This is commonly admitted by almost everyone; but it is equally true that such tests reveal, among other reactions, the heart's response to effort (8), and that such information is equally important for a functional appraisal of subjects with or without heart disease.

As any attempt to assess functional capacity or circulatory impairment must remain incomplete without electrocardiographic examination, a brief summary of its principles, pediatric considerations, and interpretation follows. In addition, a simple test for vascular tone is described, as well as methods for testing capillary mechanical function.

ELECTROCARDIOGRAPHY

The cardiac action current is led off from certain regions of the body surface to the electrocardiograph, an apparatus which works on the same principle as the galvanometer. The following standard emplacements are used: lead I, right hand and left hand; lead II, right hand and left foot; lead III, left hand and left foot; lead IV, left leg and chest (in left parasternal line and in fourth intercostal space).

The normal electrocardiogram in all leads is made up of a tracing which shows distinct movements or deflections to one or the other side of the horizontal or "isoelectric" line. These deflections occur in regular groups of five waves, each group corresponding to one cardiac cycle.

While the normal pattern of the five waves varies considerably as to amplitude and duration of deflections, there is general agreement as to the limits of normality.

The entire area through which the tracing moves is divided into 1 mm. squares. Horizontally, these squares represent units of time, the distance between two lines being equivalent to 0.04 second. Vertically, the squares represent units of voltage, each square being equivalent to 0.1 millivolt.

The five waves have been named P, Q, R, S, and T waves, and the figures 1, 2, or 3 are added to denote the respective lead, e.g., P_1 or R_3. Their deflections, called amplitude, are given in terms of millimeters or millivolts. The duration of a single wave or of wave complexes, as measured in the horizontal direction, is expressed in terms of seconds.

The P wave represents the electric potential caused by auricular contraction. The Q, R, S, and T waves are produced by ventricular action. The PR interval is the length of time needed by the excitation wave to travel from the sinoauricular node through the auricles and into the ventricular conducting system. The QRS complex, measured horizontally, gives the time required by the impulse to spread over the ventricles. The T wave originates during the retreat of the impulse. The S–T interval expresses the time between the end of the S wave and the beginning of T.

PEDIATRIC CONSIDERATIONS

The electrocardiogram is an invaluable aid in the management of heart disease in children. The test itself and its interpretation is usually left to the expert, so that the pediatrician who receives the report is often tempted merely to read and accept the final diagnosis, without ascertaining the details which led to the report. Such resignation does neither the patient nor the physician any good. A more active and more critical approach to electrocardiographic studies on the part of pediatricians seems justified, provided it is limited to those facts which can be mastered without specialized knowledge in cardiography. This limitation applies equally to the discussion that follows. For further information, the reader is referred to Nadrai's notable monograph (9), and to articles on pediatric electrocardiography (10–17).

The normal pattern of the electrocardiogram in children, as well as the typical departures from it, differs distinctly from that found in the adult. Age bears a definite relation to the pattern, the normal tracing changing continuously from birth to adolescence. Departures from the normal of diagnostic value in the adult, therefore, often have no pathologic significance in the child. Such abnormalities in adults as are due to hypertonia, coronary sclerosis, or myocardial infarction, for example, cannot be interpreted in the same way when they are found in children.

The main technical obstacle to a reliable tracing in infants and children is the difficulty in attaining standard conditions. To obtain correct results, the child should be lying down, should not be crying, and arms and legs should not be touching each other. Once the electrodes are placed, they must be carefully watched, since any movement (e.g., deep breathing, sneezing, or coughing) may dislodge them. With patience and ingenuity, however, a fair degree of relaxation may be obtained. A darkened room, a nipple or bottle, a diaper or towel covering the head, may help infants to go to sleep. If such attempts fail, rectal administration of 0.2 to 0.3 Gm. of chloral hydrate will induce relaxation, and the only effect which the drug may have is to reduce the frequency.

Only leads I, II, and III are recorded in children. Lead IV cannot as yet be accepted for routine interpretation, for the criteria of normality for this tracing in children still remain to be established (18,19). Opinions also differ as to the proper precordial region from which lead IV should be obtained (20).

INTERPRETATION

Knowledge of the normal tracings of children forms the basis for any interpretation of a child's electrocardiogram. To describe the norm for each age group briefly yet accurately is difficult. Table 49 lists the essential changes with age in the electrocardiogram of normal children, and these are illustrated by Figure 38, which shows the composite curves as drawn by Seham and Moss (16). All the values appearing in the table are averages derived from the average normal values, as reported by the authors quoted by Nadrai (9). Actual values vary within wide limits. Thus Lincoln and Nicholson (11), for instance, found that amplitudes of the P wave in lead I in preschool children fluctuate between 0.5 and 3 mm.; Burnett and Taylor (13), between 0.5 and 2 mm.; and Hecht (10), between 0.3 and 1.8 mm. The mean average in Table 49 is 1.0 mm. It seems obvious, therefore, that departures from listed normal averages need not necessarily be an indication of disease; they may possibly be merely normal variants. These wide physiologic variations must always be kept in mind when a diagnosis of heart disease is made on the basis of an electrocardiographic record, if one wishes to guard against the possibility of diagnostic error. A repetition of the test often helps to avoid this pitfall.

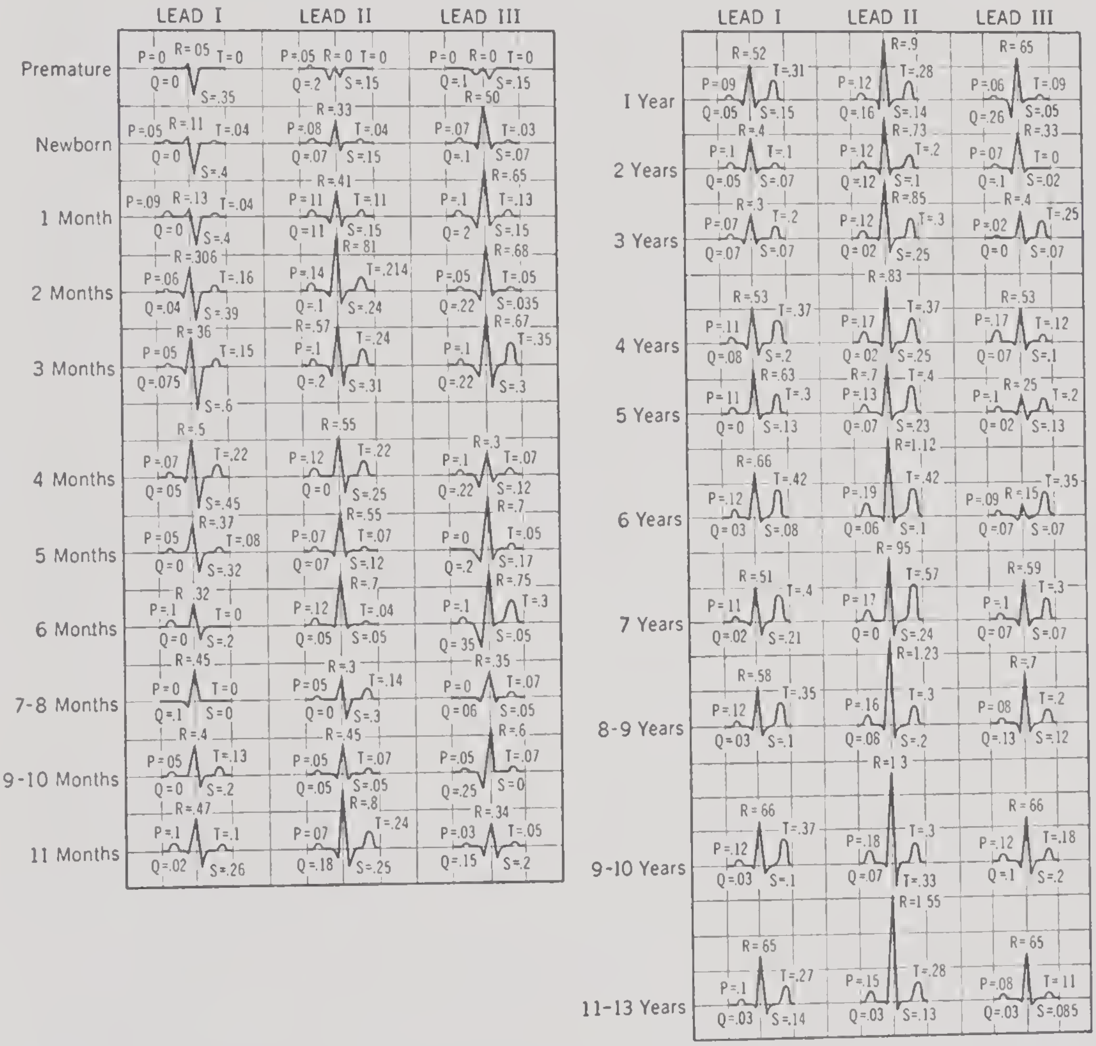

Fig. 38. Composite electrocardiographic curves of 101 children, from birth to 13 years. From Scham and Moss (16).

The electrocardiogram of normal children shows many peculiarities, and only the more significant will be given here. The P wave is always positive (upward) in leads I and II, but it may be negative or diphasic in lead III. The Q wave is of greater significance in children than in adults. Its depth decreases with age. The main deflections of the QRS complex in the majority of records are upright in

TABLE 49

Measurements of Amplitude and Duration of Electrocardiographic Cycles in Children. Variation of Normal Averages with Age and Accepted Limits of Normal

Wave, interval, or complex	Deflections	Normal in children				Pathologic in children	Normal in adults
		Newborn	1–12 mos.	2–5 yrs.	6–14 yrs.		
P	Amplitude, mm.						
	P_1	0.3	0.6	1.0	1.2	>1.5	1–3
	P_2	1.3	1.4	1.5	1.6	>2.5	
	P_3	0.5	1.0	1.2	1.5	>2.5	
	Duration, sec.	—	—	—	—	>0.09	0.08
PR	Duration, sec.	0.113	0.125	0.125	0.138	> Normal value +0.02	0.18
QRS	Amplitude, mm.	—	10–18	—	—	<7.0	5–16 (less in lead III)
	Duration, sec.	0.04	0.045	0.065	0.07	>0.09	0.1
Q	Amplitude, mm.						
	Q_1	Absent	0.3	0.5	0.4	—	—
	Q_2	1.0	1.0	1.3	0.5	—	—
	Q_3	2.6	3.6	2.2	0.9	—	—
R	Amplitude, mm.						
	R_1	2.2	7.2	6.4	6.2	—	6.2
	R_2	6.6	9.6	11.8	12.5	—	15.0
	R_3	9.0	5.9	5.5	6.4	—	5.0
S	Amplitude, mm.						
	S_1	6.2	3.1	2.9	1.7	—	1.4
	S_2	2.5	2.5	1.3	1.6	—	
	S_3	0.4	0.3	1.0	1.4	—	
ST	Duration, sec.	Insignificant, varying with heart rate					
T*	Amplitude, mm.						
	T_1	Absent–1.4	2.8	2.9	3.0	—	Half of QRS
	T_2	1.5	3.2	3.2	3.4	—	
	T_3	0.4	1.0	0.9	1.3	—	

According to Nadrai (9). * Very variable.

all three leads; in some instances, however, they may be inverted as a result of the position or form of the normal heart. Right axis deviation, i.e., an inverted QRS_1 and an upright QRS_3 is normal in premature, newborn, and young infants, in all of whom the right ventricle is preponderant. Such a deviation is also found frequently in asthenic children with a long, hanging heart. Left axis deviation, the result of a heart in transverse position, is rarely encountered in children.

The S–T segment normally runs slightly below or above the isoelectric line, the normal limit being within 1 mm. of the base line. Occasionally, this segment is absent, and the T wave rises directly from R and S. Some slight notching of P and some splitting of R and S deflections are considered normal.

The characteristics of the normal pattern in infants may be summarized as: (*1*) right axis deviation; (*2*) deep S waves in leads I and II; (*3*) deep Q waves in leads I and II; (*4*) low amplitudes; and (*5*) increased rate.

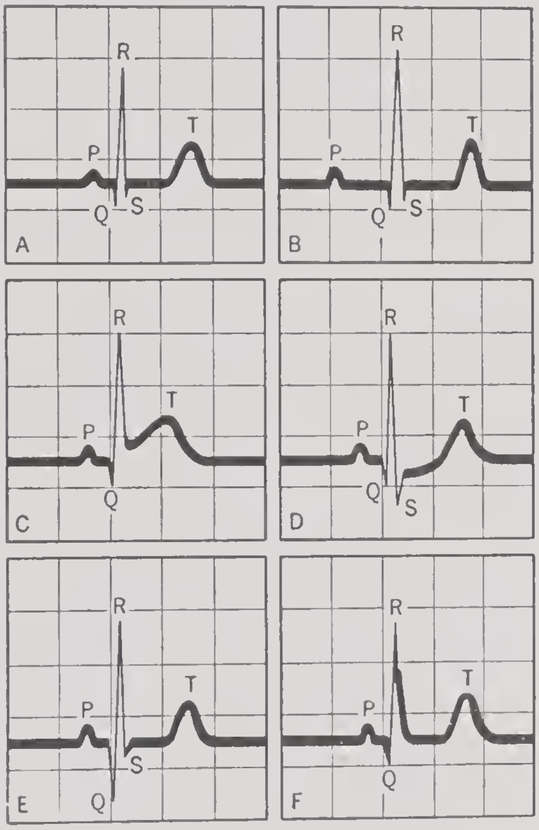

Fig. 39. Some typical abnormalities in electrocardiographic tracings, as observed in children. A: Normal cardiac cycle. B: Prolongation of PR interval (0.24 second). C: Elevation of ST segment. D: Depression of ST segment. E: Pardee Q_3. F: Notching of R wave. From Seham and Moss (16).

The most important changes in the electrocardiogram indicative of disease (Fig. 39) are in: (*1*) amplitude of deflection, either an elevation or a depression; (*2*) duration of waves or intervals, either a lengthening or shortening; (*3*) shape of contours, consisting of notching, splitting, or slurring; (*4*) direction of deflections, consisting of upward or downward inversion; (*5*) rhythm of the cardiac cycle, consisting of arrhythmia, and various types of heart block; and (*6*) heart rate, either tachycardia or bradycardia.

While the correct interpretation of all these abnormal configurations, with respect to the underlying pathologic changes in the heart and their clinical significance, calls for cardiographic experience, it is very helpful if one is familiar with the most typical abnormal patterns of children's electrocardiograms. One should also bear in mind the physiologic variations caused by heart position, respiration, and the like, which may simulate pathologic tracings.

Since certain changes in the electrocardiographic pattern reflect damage of a definite part of the heart muscle, such changes may be found in different diseases producing identical myocardial injury. The abnormal shape of one wave or one complex is practically no clue to the nature of the disease, but a combination of changes is apt to reveal a definite ailment.

Pediatric interest in electrocardiography centers on rheumatic heart disease, cardiac conditions due to infectious diseases, and congenital heart disease.

The most significant changes produced by rheumatic heart disease (Table 50) consist principally of notching of the main deflections (P, R), flattening and widening of the P wave, abnormalities in the T wave, and lengthening of the P–QRS interval. Frequently, a delayed PR interval is the only electrocardiographic sign of damage to the heart. This sign is considered most significant of rheumatic processes. Various degrees of abnormal rhythm may be present, from extrasystole to complete heart block. The diagnostic significance of the RS–T deviations is questionable.

The predominant changes observed in infectious diseases, according to Seham and Moss (16), may be listed as: (*1*) paroxysmal tachycardia, and (*2*) damage to the conduction system, which leads to all possible degrees of heart block, and is evidenced by a delayed PR interval. The characteristics of tracings obtained in diphtheric myocarditis are given in Table 50. A slur near the apex of R, combined with other bizarre forms of the QRS complex, depression or inversion of the T wave in one or all leads, and a deep Q_3 wave, are the most typical changes. In contrast to diphtheria, scarlet fever causes abnormalities similar to those of rheumatic fever (24). Conceivably, the close relationship between the scarlet fever streptococcus and the hemolytic streptococci etiologically implicated in rheumatic fever accounts for this resemblance.

TABLE 50. Characteristics of the Electrocardiographic Cycle in Rheumatic and Diphtheric Heart Disease

Rate*	Rhythm	P wave	Q wave	PR interval	QRS complex	ST interval	T wave
Rheumatic							
B or T	Sinoauricular block; rarely, atrioventricular block	Flattened; lengthened (>0.09 sec.); split, slurred, or notched; may be inverted		Lengthened ("delayed")	Slightly lengthened; split, notched in leads I, II, III; negative in lead I; high voltage	Elevation (questionable)	Flattened or negative in lead I; high in lead III; notched
Diphtheric							
ST	Partial or complete atrioventricular block		Deep in lead III		Prolonged; slurred (near apex of R); high voltage; occasionally bizzare pattern	Prolonged; depressed	Flattened or inverted; diphasic or isoelectric in lead III; diphasic or isoelectric also in leads I and II if the disease process is progressing.

According to Nadrai (9), Brackeley (21), Drawe *et al.* (22), and Swift and Cohn (23).
* B, bradycardia. T, tachycardia. S, sinus.

TABLE 51. Electrocardiographic Changes Observed in Some Noncardiac Diseases in Children

Disease	Rate	P wave	Q wave	PR interval	QRS complex	ST interval	T wave
Intracranial hemorrhage	Bradycardia	High, wide		Widened, lengthened			
Infantile eczema	Bradycardia	Wide	Deep in lead III			Sloping upward	Very high upright
Tetany	Tachycardia			Shortened (0.05–0.075 sec.)	Occasionally widened	Lengthened	High, sharply peaked
Pyloric stenosis				Lengthened	Widened		Flattened

According to Nadrai (9).

The diagnostic value of the electrocardiogram in congenital heart disease should not be overestimated. With the exception of dextrocardia, there is no characteristic pattern for any of the congenital malformations. In the majority of cases there are multiple anatomic anomalies, leading to a great variety of departures from the normal pattern (25,26a). The changes suggestive of congenital heart disease are: (*1*) extremely pronounced axis deviation; (*2*) diphasic ventricular complex; (*3*) increased, enlarged, or doubled deflections of the P wave; and (*4*) high voltage. Figure 40 shows a typical tracing; the difference between this pattern and that found in rheumatic conditions may easily be seen. But occasionally it is almost impossible to decide whether the electrocardiographic changes are the result of acquired or congenital heart damage.

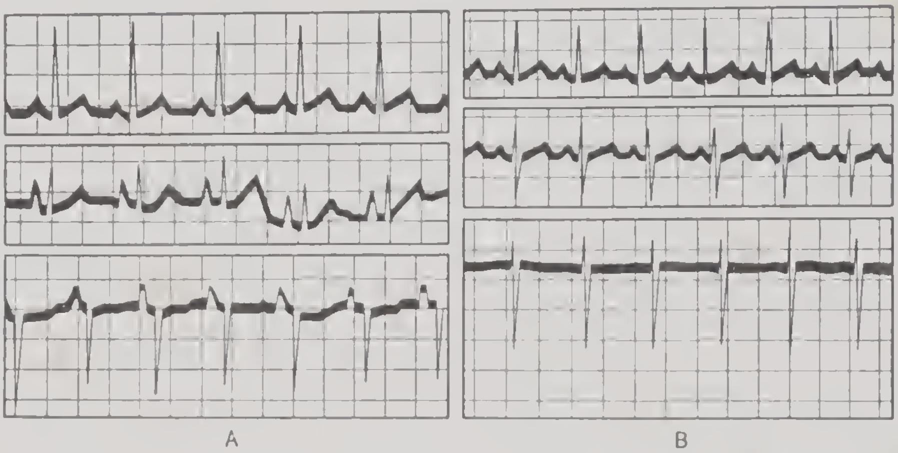

Fig. 40. (A) Electrocardiogram of 4 month old infant. Anatomic diagnosis: congenital malformation of the heart; cor triloculare; complete atresia of the tricuspid and pulmonary valves; complete absence of right ventricle; anomalous patent ductus arteriosus; patent foramen ovale; hypertrophy of right auricle and left ventricle. *Interpretation of electrocardiogram*: normal sinus mechanism; a rate of 150 per minute; a PR interval of 0.12 second; left axis deviation. (B) Electrocardiogram of a 5 week old infant. Anatomic diagnosis: congenital malformation of the heart; hypoplasia of right ventricle and tricuspid valve; patent ductus arteriosus; patent foramen ovale; atresia of pulmonary artery. *Interpretation of electrocardiogram*: normal sinus mechanism; a rate of 140 per minute; a PR interval of 0.12 second; left axis deviation. From Taussig (26b).

Abnormal deflections have also been found in a number of noncardiac diseases in children, as may be seen from Table 51.

TWO-STEP TEST OF CIRCULATORY FITNESS

This test is based on the facts that both blood pressure and pulse rate increase during physical exertion, and that the rates of increase and return to normal depend on heart function, nervous regulation, and vascular tone. Abnormally prolonged reactions to a standard exercise are considered significant of impaired circulatory efficiency, particularly of myocardial weakness. The test presents no difficulty for any child over 4 years of age.

PROCEDURE

The method described is that of Master (27). The blood pressure and pulse rate are taken and recorded while the child is at rest (sitting or standing), the lowest of several readings being considered normal. The child is then asked to go up and down two 9 inch steps for as long as 1½ minutes. Table 52 gives the number of climbs on the steps considered standard for age, sex, and weight. After the exercise and a rest period of 2 minutes, pulse rate and blood pressure are again taken. The difference between the values obtained before and after exercise is the criterion of a normal or abnormal response.

TABLE 52
Standard Number of Ascents for Two-Step Test

	Males			Females		
	Age, years			Age, years		
Weight, lbs.	5–9	10–14	15–19	5–9	10–14	15–19
40–49	35	36	—	35	35	33
50–59	33	35	32	33	33	32
60–69	33	31	31	31	32	30
70–79	28	32	30	28	30	29
80–89	26	30	29	26	28	28
90–99	24	29	28	24	27	26
100–109	22	27	27	22	25	25
110–119	20	26	26	20	23	23
120–129	18	24	25	18	22	22
130–139	16	23	24	16	20	20
140–149	—	21	23	—	18	19
150–159	—	20	22	—	17	17
160–169	—	18	21	—	15	16
170–179	—	—	20	—	13	14
180–189	—	—	19	—	—	13
190–199	—	—	18	—	—	12

According to Master (27).

INTERPRETATION

A difference of 10 or less in either pulse rate or blood pressure is considered negative or normal.

An increase in either value above 10 is interpreted as a positive or abnormal response.

The efficiency of the circulation may be expressed in terms of a percentage of normal. For instance, if a child is able to perform 13 instead of 17 climbs, the efficiency is $^{13}/_{17}$, or 77 per cent. If the child cannot tolerate the standard number of climbs for his age, sex, or weight, another test, with fewer climbs, is tried, until the limit of work is found which he can perform with abnormal reactions.

Abnormal reactions are found in subjects with impaired circulatory function. Since in children heart function is the predominant factor governing circulatory capacity, an abnormal result is generally considered to be a sign of impaired myocardial function.

The test is particularly useful in detecting subclinical stages of myocardial damage, and therefore may be used during convalescence after diphtheria and scarlet fever, and in clinically inactive cases of rheumatic heart disease (28). However, there is no quantitative relationship between the results of the test and the extent of cardiac damage; constitutional neurovascular asthenia alone may suffice to produce positive responses (7). More accurate information on the character of myocardial weakness is provided by the test which follows.

ELECTROCARDIOGRAM AFTER STANDARD EXERCISE

As outlined by Master *et al.* (29a,8), electrocardiographic tracings are made after a subject has undergone the two-step test, as outlined above. Normally, the standard amount of exercise does not produce abnormal electrocardiographic patterns. If, however, the standard exceeds the tolerance of the heart, abnormal changes in the electrocardiogram may appear. Only the standardized amount of exercise may be used; exercise in excess of this amount may be followed by a pathologic electrocardiographic response even in healthy children.

As the physical activity leads to a transient anoxia of the myocardium (8), the electrocardiographic changes after exercise in children resemble those observed in coronary insufficiency in adults.

PROCEDURE

A control electrocardiogram is taken before the exercise is started, the test is performed using the technic described on page 306, and three more electrocardiograms are taken. One is done immediately on cessation of the exercise, the second 3 minutes later, and the third 8 minutes after the first. It is advisable to keep the electrodes strapped to the child's arms and legs during the actual performance of the exercise, so that the electrocardiogram can be taken immediately thereafter.

INTERPRETATION

Normally, control and exercise records are identical.

The response is considered abnormal if one of the three tracings shows changes from the control pattern. The commonly observed changes (29a–b) are: (*1*) RST complex depressed more than 0.5 mm. below the base line; (*2*) positive T wave changed to a flat (isoelectric) or inverted T wave in leads I and II, or a negative T wave changed to a positive one; and (*3*) multiple premature beats, widening of the QRS complex, deepening of the Q wave, prolonged PR interval, or heart block. Such abnormal tracings may be obtained in subjects whose response to exercise is normal, as judged by the reaction of pulse rate and blood pressure.

Abnormal tracings are obtained in: (*1*) cardiac disorders, such as valvular, coronary, or myocardial disease, and in congenital malformation of the heart; (*2*) noncardiac conditions affecting the circulatory capacity, such as infections of the upper respiratory tract, lung disease, or severe gastroenteritis; and (*3*) neurocirculatory asthenia (the "effort syndrome") leading to diminished cardiac output, reduced oxygen saturation of the blood, and anoxia of the heart muscle (8).

Results must be evaluated cautiously. Positive (i.e., abnormal) findings in the electrocardiogram after the two-step test are of greater significance in patients with a known history of cardiac disease than in patients with neurocirculatory asthenia. Negative results, on the other hand, do not rule out the possible presence of functional weakness of the heart or of organic damage. When the control electrocardiogram reveals deviations of the type that can be expected in response to standard exercise, the test should not be performed.

TEST OF VASCULAR TONE

The effect of gravity upon the blood in the arterial vessels brings into play compensatory changes in the vascular tone when the body is held erect. Blood pressure and pulse rate are higher when the subject is standing than when he is lying down. This increase in blood pressure is a sign of the vascular reaction to the changed position. When the tone of the peripheral vessels is inadequate, the rise in blood pressure is slighter, and to offset this deficiency there

TABLE 53

Grading and Scoring the Results of Test for Vascular Tone in Children

A. 4–6 years

Pulse rate on reclining		Pulse rate increase on standing, beats/min.:					Systolic blood pressure on standing	
Beats/ min.	Score, points	0–13	14–23	24–33	34–44	45–54	Change in mm. Hg	Score, points
		Score, points						
65–77	3	3	3	2	1	0	Rise of 8 or more	3
78–90	3	3	2	1	0	−1	Rise of 1–7	2
91–103	2	3	2	0	−1	−2	No rise	1
104–117	1	2	1	−1	−2	−3	Fall of 1–4	0
118–131	0	1	0	−2	−3	−3	Fall of 5 or more	−1
132–145	−1	0	−1	−3	−3	−3		

B. 6–10 years

Beats/ min.	Score, points	Beats/min.:					Change in mm. Hg	Score, points
		0–11	12–21	22–30	31–39	40–48		
59–69	3	3	3	2	1	0	Same as in A	
70–80	3	3	2	1	0	−1		
81–91	2	3	2	0	−1	−2		
92–102	1	2	1	−1	−2	−3		
103–113	0	1	0	−2	−3	−3		
114–124	−1	0	−1	−3	−3	−3		

C. 10–14 years

Beats/ min.	Score, points	Beats/min.:					Change in mm. Hg	Score, points
		0–10	11–18	19–26	27–34	35–42		
50–60	3	3	3	2	1	0	Rise of 8 or more	3
61–70	3	3	2	1	0	−1	Rise of 2–7	2
71–80	2	3	2	0	−1	−2	No rise	1
81–90	1	2	1	−1	−2	−3	Fall of 2–5	0
91–100	0	1	0	−2	−3	−3	Fall of 6 or more	−1
101–110	−1	0	−1	−3	−3	−3		

From Netzley (30).

is a greater increase in pulse rate. A considerable rise in pulse rate, coupled with insignificant changes in blood pressure, suggests poor vascular ability to counteract the effect of gravity when standing.

PROCEDURE

The method described is that of Netzley (30). Brachial blood pressure and pulse rate are recorded after the child has rested for 5 minutes in supine position. The child is then asked to stand up in a relaxed position, and after 2 minutes blood pressure and pulse rate are taken again. The results are computed and scored as shown in Table 53.

INTERPRETATION

In healthy children who show good endurance and have no history of fatigue, the scores obtained are: (*1*) no grading lower than 4 points; (*2*) all gradings between 4 and 9 points; and (*3*) average normal, 6.4 points. Scores below 5 are considered poor; they are found in children with chronic fatigue and poor endurance. Included in this group are children with nervous irritability and physical hyperactivity, all of whom show scores from 2.5 to 4.5.

All minus gradings suggest that the circulatory weakness is caused by a true pathologic condition, particularly cardiac insufficiency.

In a subject whose pulse rate is abnormally high when he is reclining, the rise in blood pressure after standing is more significant than the total score, which obviously must be low. But any rating below 1 either in pulse rate or in blood pressure is indicative of deficient circulatory power.

CAPILLARY RESISTANCE TESTS

Weakness of the endothelial lining, whatever its cause, leads to increased capillary fragility and reduced capillary tone. When the capillary membrane becomes so weakened that it cannot withstand the normal intracapillary pressure, blood penetrates the injured vascular wall, with resultant small hemorrhages (petechiae). In the absence of spontaneous petechiae or purpuric spots, the condition of the capillaries can be ascertained by means of tests which measure the capillary resistance to positive or negative pressure applied to a skin area open to observation. Abnormally fragile capillaries yield to slighter pressure than normal vessels. In all cases, the criterion is the appearance of petechiae.

According to Wiemer (31) and Dalldorf (32), the factors which are known to influence capillary fragility fall into two groups: those damaging the endothelium directly, and those affecting capillary resistance indirectly. The first group includes (*1*) poisons, such as opium, neoarsphenamine, carbon monoxide; (*2*) toxins, as in scarlet fever, diphtheria, polyarthritis; (*3*) metabolic products, as in uremia or acetonemia; (*4*) scurvy. The second group includes (*1*) physiologic variations, due to age, menstruation, or season; (*2*) endocrine diseases, such as exophthalmic goiter; (*3*) diseases of the spleen and reticuloendothelial structures.

TOURNIQUET TEST

Among the various modifications of the original Rumpel–Leede method (33,34), that described by Hess (35) is the most practical one.

A tourniquet or blood pressure band, its width adapted to the child's age and size, is placed about the arm, 2 to 3 inches above the elbow. The pressure is increased until the fore arm becomes cyanosed and the radial pulse is almost, but not entirely, obliterated. Pressure is maintained at this level for 3 minutes. In infants the pressure is usually raised to 90 mm. mercury.

The appearance of petechial spots on the forearm or in the bend of the elbow is a positive reaction.

The significance of this test is doubtful, since with advancing age a steadily increasing number of normal children have a positive reaction. According to Brock and Malcus (36), the percentage of positive reactors, as determined by various authors is:

Per cent	Age
0–16	1–3 months
37–48	4–12 months
71	1–1½ years
69	1–6 years
73	7–14 years

As a result, the tourniquet test has been widely abandoned, and the suction test is used instead.

QUANTITATIVE TOURNIQUET TEST

This test has been evolved by Göthlin (37). With a rubber tourniquet, a mercury manometer, Politzer rubber syringe, and a compressor for the syringe, the response to pressure of 30, 50, and 65 mm. mercury is ascertained. Göthlin insists that the test be performed at infradiastolic pressure, and that the duration of pressure be extended to 15 minutes.

The test starts with a 15 minute period of compression at 50 mm. If the result is negative, the test is repeated at a pressure of 65 mm. The result is definitely negative if no petechiae appear. When the result is positive at 50 mm., the test is repeated on the other arm with a pressure of 35 mm.

Positive responses are graded as: (*1*) Appearance of petechiae at 65 mm. mercury. (*2*) Appearance of not more than 6 petechiae at 50 mm. (*3*) Appearance of more than 6 petechiae at 50 mm., but not at 35 mm. (*4*) Appearance of petechiae at 35 mm.

FLICKING TEST

As described by Jones and Tocantins (38), a tourniquet, a broad rubber band, or a piece of large-sized rubber tubing is placed around the arm, as in the Rumpel-Leede test. After 4 minutes, with the tourniquet still in place, the examiner flicks his middle finger 3 or 4 times against the distended veins of the forearm, or anywhere between these veins.

The appearance of petechiae in the areas that have been flicked is considered a positive reaction. The percentage of positive reactions obtained in healthy children is considerably smaller than in the Rumpel–Leede test.

SUCTION TEST (CAPILLARY RESISTANCE TEST)

The test was devised by Hecht (39). The procedure described is that of Dalldorf (32), as modified by Brown (40). A resistometer, consisting of a small hand pump, a vacuum gage registering in centimeters of mercury, and a stopcock, is used for the quantitative application of suction. The instrument is connected by vacuum rubber tubing to a glass suction cup, 1 cm. in diameter, with the edge turned out at a right angle to a breadth of 8 mm.

The glass cup is applied to the forearm about 2 cm. distal to the antecubital fossa, after the skin has been moistened with water.

By pulling back the handle of the pump, negative pressure is applied. A given pressure, as indicated by the gage, is maintained for 1 minute, the cock is then turned so that the suction is released, and the cup is removed. If at least 2 petechiae have not been produced, the test is repeated on adjacent skin areas with negative pressure increased by 5 cm. mercury. This is repeated until petechiae appear.

TABLE 54
Average Normal Capillary Resistance for Age

Age, years	Pressure, cm. Hg
Newborn	50
1–2	33
3–4	30
5–7	27
8–10	24
11–15	28
16–20	26
21–45	23
46–60	22

If the first test produced petechiae, it is repeated with negative pressures decreased by 5 cm. mercury each time. The lowest negative pressure producing a positive reaction is considered as the capillary resistance.

In evaluating the results, age and season must be taken into account. The average normal capillary resistance in children varies between 25 and 35 cm. mercury, depending on the child's age. Normal resistance is higher during the first year of life than in older children. Table 54 gives the average normal values for age.

Seasonal variations may amount to as much as 10 cm. of mercury. The highest resistance is found during the summer, the lowest during late winter (41).

Results are considered abnormal when the resistance is lower than the average normal value for the age of the subject. Decreased capillary resistance, i.e., increased fragility, is a characteristic finding in scurvy (page 284), thrombocytopenic purpura (42), and at the onset of scarlet fever. Resistance as low as 5 mm. of mercury has been found. In rheumatic fever, colitis, and measles, the decreases observed have been milder and less constant.

VENOM TEST

The test, as described by Park, Rosenthal, and Erf (43), consists of the intradermal injection of 0.1 cc. of 1:3,000 solution of standardized mocassin venom (commercially available as venomin), with a control injection of 0.1 cc. of physiologic sodium chloride solution. The site of injection is examined after 1 hour. Capillary rupture around the site of injection is a positive reaction. The ecchymotic area may be small, or it may involve the skin far beyond the point of injection. Delayed reactions, developing 12 or more hours after injection, are of no clinical significance. Absence of hemorrhagic reactions is interpreted as a negative result.

A single positive result is a sign of a state of purpura, or of the existence of a local or generalized capillary fragility. The results of successive tests have a prognostic value. Continued positive reactions suggest persistence of the bleeding tendency and failure of therapeutic measures. Reversal of positive to negative results indicates that the abnormal capillary fragility has begun to subside and has begun to respond to treatment. The test has proved very helpful in the management of thrombocytopenic purpura.

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CHAPTER X

Immunologic Tests

SKIN TESTS FOR IMMUNITY

The substances used for such tests are toxic products of the disease-producing agents (1a). Individuals who have enough antibodies in the skin to neutralize the test dose of the toxin suffer no essential damage to the skin and produce no visible reaction, i.e., they are immune. In the absence of immunity, there is a positive reaction; such individuals are susceptible to the disease being tested for.

Positive responses to these skin tests are of the delayed type, requiring about 24 hours to develop. The occasional cases in which intradermal injection of diphtheria toxin also produces an immediate reaction, in all probability reflect allergy to the test substances.

DIPHTHERIA TOXIN TEST

The Schick test (2) ascertains the response of the skin to intracutaneous injection of a small amount of diphtheria toxin. An inflammatory reaction at the site of injection is a sign that there is very little circulating antitoxin, or none at all. Absence of a reaction points to a level of antitoxin which is currently believed to be at least $^1/_{30}$ unit per cubic centimeter of serum.

The true positive reaction is of the delayed type. The allergic reaction, or pseudoreaction, which occurs occasionally, appears immediately; this reaction is due to hypersensitivity to foreign proteins contained in the toxin. A control injection of heated toxin solution is used in order to recognize the presence of a pseudoreaction.

PEDIATRIC CONSIDERATIONS

The greatest usefulness of the Schick test is (*1*) in ascertaining the effectiveness of active immunization in an individual child or in mass surveys, and (*2*) when the differential diagnosis of diphtheria depends on the demonstration of susceptibility or immunity.

Mass surveys of healthy individuals with this test have shown that the frequency of positive reactions varies with age (2). At birth, 93 per cent of infants have antibodies acquired by transfer from their mothers, and therefore give a negative reaction. At the age of 6 months, up to 50 per cent of the infants tested show positive reactions; between the ages of 8 months and 3 years the majority become positive reactors, and are therefore susceptible to diphtheria. Immunity increases thereafter; between the ages of 5 and 15 years only about 50 per cent of the children give positive reactions, and 90 per cent of adults give negative reactions.

PROCEDURE

The skin on the flexor surface of both forearms is cleansed with 95 per cent alcohol, ether, or acetone, and allowed to dry. On one arm 0.1 cc. of the commercially available diphtheria toxin is injected intracutaneously, using a 25–27 gage, ½-inch long needle. A wheal or bleb must form as a result of the injection. The control injection of 0.1 cc. of heated toxin dilution is made on the other arm in the same manner.

Syringes used for tuberculin tests should not be employed.

Results are read on the first or second day after injection, and on the fourth day. A positive reaction consists of an area of redness and infiltration around the site of injection; in extremely strong reactions there may even be vesiculation.

True positive reactions appear within 24 hours, reach their greatest intensity on the third or fourth day, and thereafter fade gradually, leaving a brownish pigmented area, with or without desquamation.

If reaction to the control injection occurs, it develops more rapidly than the true reaction, reaching its greatest intensity in 48 hours and fading or disappearing completely on the third or fourth day, at a time when the true reaction is at its height.

INTERPRETATION

A positive reaction, with a negative control, indicates susceptibility to diphtheria. It is assumed that the stronger the skin reaction is, the lower the antitoxin titer in the patient's serum.

A negative response, with a negative control, is a sign of immunity against diphtheria.

An allergic reaction or pseudoreaction may be observed in addition to, or in the absence of, true positive reactions. If both appear (combined reaction), the reaction to the control injection occurs more rapidly and is weaker than that on the other arm, where the true reaction is superimposed on the pseudoreaction. A combined reaction reveals the presence both of susceptibility to diphtheria and of protein allergy.

Equal intensity and rate of development of the reactions on both arms, with the highest point being reached in 48 hours, may be interpreted as a pseudoreaction. In such cases, immunity to diphtheria toxin is associated with protein allergy.

The significance of the various types of response to the Schick test is shown in Table 54A.

TABLE 54A
Interpretation of Responses to Schick Test

Visible reactions on			
Left arm (toxin)	Right arm (control)	Reading	Interpretation
(*1*) Positive	Negative	Positive	Not immune; not allergic
(2) Negative	Negative	Negative	Immune; not allergic
(*3*) Positive, larger than *1*	Pseudopositive	Combined	Not immune; allergic
(*4*) Pseudopositive	Pseudopositive	Pseudo	Immune; allergic

From Boyd (1b).

When extremely strong responses, such as "bullous reactions" (13), are obtained in patients with acute febrile diseases, interpretation is doubtful and the child has to be retested after recovery.

Differentiation between pseudoreactions and true reactions is an essential factor in the evaluation of the Schick test. Usually, early observation of the reaction prevents a wrong interpretation. in children older than 10 years and in adults who are to be immunized with toxoid.

DIPHTHERIA TOXOID TEST

This test was originally proposed by Zoeller (3). The description that follows is the modification of Moloney and Fraser (4). Although actually the test demonstrates hypersensitivity (allergy) rather than immunity, its close practical relation to the Schick test makes the discussion of it here logical.

Potential allergic reactions to immunization with toxoid may be detected more reliably with this intradermal test than with the control injection of the Schick test. In early childhood sensitivity to diphtheria toxoid is very rare; the test is therefore most useful in older children, and particularly in adults who are to be immunized with toxoid.

The test consists of intradermal injection of 0.1 cc. of a 1:100 dilution of diphtheria toxoid. In a positive reaction there is an erythema; if the reaction is very strong, there is also infiltration around the site of injection. The maximum point of the reaction is reached after 24 hours.

For practical purposes, it is recommended that the Schick test and control be done first; the toxoid test is then performed only on persons with a positive reaction to the Schick test. The results of this second test determine the mode of active immunization.

Negative and mildly positive reactors to the Moloney test may be immunized with toxoid according to the routine procedure. Moderately and strongly positive reactors should receive an intracutaneous injection of 0.1 cc. of toxoid dilution (1:100) every week for 3 consecutive weeks. A second Schick test is done 6 months later; if the reaction is still positive, a further series of weekly injections is given, but subcutaneously, not intracutaneously.

SCARLATINAL TOXIN TEST

The Dick test (5) is an intracutaneous test for immunity to the toxin of the hemolytic streptococcus causing scarlet fever (Str. scarlatinae). The reaction to the test probably depends on whether or not the toxin of the test dose is neutralized by the antitoxin present in the skin. Insufficient antitoxin will cause positive reactions, while a high antitoxin titer will inhibit any cutaneous response to the injected toxin. The reaction to the Dick test is of the delayed type.

PEDIATRIC CONSIDERATIONS

The Dick test is the best available means for establishing the need for preventive measures against scarlet fever, both in the prophylactic management of an individual exposed to the disease and in the control of epidemics. The information provided by the Dick test is essential for the proper choice of restrictive rather than of immunologic measures.

According to Dick and Dick (5), the immunity to scarlet fever, as judged by the Dick test in normal children, is not related to age, "except indirectly as this factor influences the frequency of contact with other people."

PROCEDURE

The middle part of the flexor surface of both forearms is cleansed with 95 per cent alcohol, ether, or acetone, and allowed to dry. The test dose of scarlatinal toxin, 0.1 cc., is injected intradermally on one arm, and a control injection of heated toxin on the other. Both toxin preparations are commercially available. The reaction is read after 24 hours. Redness around the site of injection, covering an area at least 1 cm. in diameter, is interpreted as a positive reaction. Even a slight reddish discoloration constitutes a positive reaction. The reddened area is never indurated. The color fades without any residual pigmentation very soon after it has reached its maximum, 18 to 24 hours after injection.

Syringes used for tuberculin tests should not be used.

INTERPRETATION

A positive reaction indicates susceptibility to scarlet fever. Absence of a reaction (negative reactors) is a sign of immunity to the disease.

The percentage of negative reactors who are nevertheless susceptible to scarlet fever is higher than the percentage of subjects who give a negative reaction to the Schick test but eventually contract diphtheria.

Pseudoreactions, i.e., positive control reactions, are uncommon and easily distinguished from true reactions. They develop slowly and last longer than true reactions, fading only after the second or third day after injection.

Most patients with scarlet fever become Dick-negative during convalescence, occasionally even toward the end of the actual disease. Newborn infants are almost always negative reactors, due to a natural immunity which extends into the second half of the first year; thereafter, the majority of all children become Dick-positive.

BLANCHING TEST

In the Schultz-Charlton test (6), the principle of the Dick test is applied in reverse. In the latter the toxin is injected into the skin to ascertain the antitoxic potency, in the former antitoxin is introduced into the patient's skin in order to detect the presence of toxin. If injected intradermally into an area of scarlet fever rash, the specific antitoxin will neutralize the toxin within the zone of injection and produce a blanching of the rash.

The commercially prepared antitoxin, which is derived from horse serum, may give serum reactions or result in sensitization to horse serum. These undesirable effects can be avoided by using scarlet fever convalescent serum, which should be obtained from children or adults during or after the third week following the onset of the disease. Other effective blanching agents are globulin extracts of pooled human serum, now available from blood banks, and placental globulin. Because of its irritating effect, the latter is not recommended for routine use.

PROCEDURE

Convalescent Serum. Into an area of most intensive rash, preferably on the abdomen or chest, 0.1 cc. is injected intradermally. The site of injection is examined for blanching 18 to 24 hours later.

To prepare the serum, 5 cc. of blood are withdrawn from a patient known to have scarlet fever during or after the third week of illness, and the blood is transferred into a centrifuge tube. After 1 hour, the clot is carefully separated from the wall of the tube, and the tube is centrifuged. The supernatant serum is aspirated into a 1 cc. syringe. The entire procedure is carried out under aseptic conditions.

Antitoxin. The test dose of 0.1 cc. of antitoxin is injected intradermally.

Globulin Extracts. Karelitz and Stempien (7) recommend the intracutaneous injection of 0.2 cc. of extract.

INTERPRETATION

Blanching of the rash around the site of injection, usually lasting until the rash subsides, is considered a positive reaction. Such a reaction is pathognomonic of scarlet fever. The reliability of the test decreases with the duration of the rash. Blanching is rare when a rash has been present for more than 3 days.

It has been suggested by Dick and Dick (5) that the test is less specific when convalescent serum is used as the antitoxic agent. They found that nonscarlatinal rashes, such as the exanthem of German measles, may be blanched if the serum is taken from individuals who had acquired antibodies against other diseases as well as against scarlet fever.

REVERSE BLANCHING TEST

If the exanthem of a patient with an equivocal scarlatinal rash is blanched by the serum of a child recovering from an undiagnosed rash, one may retrospectively diagnose the disease as scarlet fever, particularly if the donor's history does not reveal a previous scarlatinal infection which might be responsible for the antitoxic potency of his serum (8).

PEDIATRIC CONSIDERATIONS

The test can only be used in hospitals where patients with unquestioned scarlet fever rash are easily available as test objects, and its practical value is limited to the late diagnosis of doubtful cases, held in quarantine as a precaution.

PROCEDURE

The method described is that of Goldberg and de Hoff (8). From a patient whose rash cannot be clinically diagnosed with certainty, 5 cc. of blood are withdrawn 3 times: sample 1 on entry into the hospital (first week of illness), sample 2 on the fourteenth day after onset of illness, and sample 3 on the twenty-first day after onset. The serum is separated from each sample as soon after collection as possible, and kept in labeled bottles in the refrigerator until the third sample is collected. With 0.2 cc. of each serum sample, 3 blanching tests are performed on a patient having the heavy rash of known early scarlet fever. A fourth test is done with specific antitoxin.

INTERPRETATION

A positive reaction to sample 1 is strong evidence that the child does not have scarlet fever, since the blanching power in serum obtained during the first week of illness cannot be due to the child's current illness and must be attributed to a naturally high antitoxin titer.

Only positive blanching reactions obtained with samples 2 or 3 support a diagnosis of scarlet fever.

Only serum from patients with an intense rash will yield reliable results. It is not so much the severity of the possible scarlet fever as the intensity of the rash which affects the serum's blanching power.

SKIN TESTS FOR HYPERSENSITIVENESS

The chief difference between skin tests for immunity and those for hypersensitiveness or allergy is that immunity tests reveal physiologic changes in the subject's susceptibility to toxic agents, so that normal individuals may be positive or negative reactors, while allergy tests, when they yield a positive result, reveal a pathologic condition.

Allergy tests may be divided into (*1*) tests employing allergens not related to any living infectious agent, and (*2*) tests using allergens related to living infectious agents, among them bacterial allergens.

The skin reactions to the first group are of the immediate type, i.e., full development of the reaction within less than 30 minutes. The most important form of hypersensitiveness which can be demonstrated immediately by skin tests is "atopic" allergy. The skin response to bacterial allergens, however, is of the delayed type, as, for example, in the tuberculin test.

A. Allergies Unrelated to Living Infectious Agents

TESTS FOR ALLERGIES IN ECZEMATOUS CONDITIONS

Allergens which may be the cause of the skin manifestations are brought into contact with the skin tissues by cutaneous or intradermal application. While a positive skin reaction indicates hypersensitiveness to the respective allergen, it does not necessarily mean that the particular allergy is the cause of the clinical condition.

The most important allergens are: (*1*) vegetable and animal food proteins; (*2*) animal emanations, such as epithelial substances, danders, and feathers; (*3*) vegetable emanations, such as pollens; (*4*) dusts; (*5*) fungi or molds; (*6*) drugs; (*7*) bacterial proteins; and (*8*) contact allergens.

PEDIATRIC CONSIDERATIONS

A discussion of the immunologic problems involved in the diagnosis of allergic skin manifestations in infancy and childhood is beyond the scope of this book. However, the indications for and the significance of skin tests, insofar as they are of interest to the pediatrician, seem worthy of comment. The following brief survey is based entirely on the authoritative presentations of Hill (9) and of Sulzberger (10), to which the reader is referred for more complete information.

All the allergic skin manifestations in children that lend themselves to a successful use of diagnostic skin tests are forms of infantile eczema. Four groups of eczematous eruptions may be distinguished: (*1*) seborrheic dermatitis, (*2*) eczematoid fungus infections, (*3*) contact dermatitis, and (*4*) atopic dermatitis. The last two are the allergic dermatoses.

In seborrheic dermatitis no allergens are etiologic factors in the development of the skin condition. Fungus infections can be identified by skin tests which reveal hypersensitivity to one of the infecting organisms (page 339). The eczema in contact dermatitis is the response of the sensitized epidermis to contact with a nonprotein allergen. Patch tests with the suspected allergens help to establish the diagnosis.

Atopic dermatitis occurs only in "atopic" children, i.e., children who are hypersensitive to certain proteins to which normal individuals are not susceptible. According to Hill (9), "atopy is the predisposition to the development of hypersentivity, usually to substances of protein nature, more rarely to non-protein." Food and environmental allergens constitute the protein substances which produce hypersensitivity on contact with an atopic individual. Ratner (11a) points out the surprising fact that hypersensitivity to egg white may be present in atopic infants who have never eaten eggs.

In children, considerable difficulty is encountered in differentiating between the various forms of eczema on the basis of their morphologic appearance. In infants, for example, most eczemas are of the mixed type, presenting the clinical criteria of any combination of the four typical forms mentioned above. Moreover, the dermatologic changes, even in uncomplicated cases of atopic or contact types of eczema, do not always appear in their characteristic form.

Skin tests have been used on a large scale in an attempt to overcome these diagnostic difficulties. But the results obtained in infants and children may be somewhat disappointing. Positive reactions to protein substances and environmental allergens, considered as typical for the atopic form of eczema, have been demonstrated in numerous cases which showed all the clinical criteria of nonatopy. The number of allergens that need to be used in testing children is relatively small. Hill states (9): "One should test with all the foods they are eating or are likely to eat in the near future and with common environmental allergens to which they may be exposed." If this principle is adhered to, the tests can be performed conveniently in one or two sessions.

As to the significance of the test results, the pediatrician should bear in mind their limited diagnostic value. Whether skin tests may be dispensed with completely because of this limitation is still a matter of controversy. One may summarize the pros and cons as follows: (*1*) Skin tests in infants and children present many pitfalls and are far from accurate. (*2*) A positive skin test does not necessarily mean that the allergen in question is responsible for the eczematous condition. (*3*) Skin tests should be used to confirm the clinical diagnosis. (*4*) Skin tests may provide information as to the cause, and therefore give a clue to the best treatment. (*5*) In most cases, infantile eczema can be managed without resort to skin tests (11b), and in young children the treatment of all the different forms of eczema is often identical (10).

PROCEDURES

For Contact Type Dermatitis. Hill (9) uses patch tests exclusively. A patch consists of a 1½ inch square of adhesive tape, containing in its center a cellophane disk. The substance to be tested is placed in the middle of the disk, and the patch is applied on the subject's back or upper arm, preferably near the area of dermatitis. If the suspected allergen is a liquid, blotting paper is saturated with the allergen and used instead of the cellophane disk. Substances tested include wool, feathers, silk, egg white, and inhalants. The patches are removed after 24 to 48 hours, and the reactions are observed. A reproduction of the original skin lesion, corresponding to the size of the disk of cellophane or blotting paper, is considered a positive reaction. Occasionally, the reaction may be delayed for several days.

For Atopic Dermatitis. *Scratch Tests.* It is advisable to start with these tests. For each material to be tested, a scratch is made on the unaffected skin surface of the child's back, forearm, or anterior aspect of the thigh, after cleansing the site with 95 per cent alcohol. A borelike scarifier (9), producing circular shaped scratches, may be used to achieve scratches of uniform length. A drop of 0.05 *N* sodium hydroxide is placed on each scratch, and a different allergen powder is rubbed with a toothpick into each drop but one. The scratch with the drop of sodium hydroxide only serves as control.

Food and environment allergens should be tested. The following food allergens are listed in the order of their etiologic importance during early childhood (9):

Egg white
Milk
Wheat
Oats
Barley
Beef
Potato
Haddock
Tomato
Rice
Corn
Spinach
Pea
Carrot
Orange
Codfish
Chicken

With the exception of egg white, which should always be included, only the foods which are eaten by the child should be selected for testing.

The environmental allergens are feathers, house dust, wool, and silk.

Scratch tests with all the required allergens can be accomplished in two sessions.

Positive reactions, consisting of a growing redness around the scratch, appear within 30 minutes, and are identified as such by comparison with the control. Wheal reactions are very rare in early childhood.

Intracutaneous Tests. These are a supplementary method of testing. They are almost 100 times more sensitive than the scratch tests, and are used only with those allergens which give a negative result in the scratch test. Milk should always be included, egg white as a rule omitted. Extracts of the various allergens are commercially available.

Not more than 0.02 cc. of the liquid allergen is injected into the cleansed skin of the forearm, and the local response is observed for about 20 minutes.

In negative reactions, the original papule caused by the injection, and any traumatic irritation, disappear within a few minutes, without the appearance of any further reaction.

In positive reactions there is a gradual increase in the size of the original papule, and/or a distinct redness of the area around the site of injection. Wheal formation is not as frequent in young children as in older individuals. A negative reaction to a control injection of diluent increases the significance of positive reactions.

"Passive Transfer" Test. An indirect method of testing for hypersensitivity makes use of the fact that serum antibodies (reagins), when transferred into the skin of a nonreactor, produce local sensitiveness. This is known as the Prausnitz-Küstner reaction.

To perform the test, 0.05 to 0.1 cc. of serum from the patient to be tested is injected intracutaneously into each of various sites on one or both forearms of another individual. The site of each injection is marked with ink. If reagins are present in the patient's serum, a zone about 2 inches in diameter around each point of injection becomes sensitized. After 24 to 48 hours, the areas can be tested by intracutaneous injection of the allergens selected, as in the direct procedure. Areas giving a negative reaction may be used repeatedly. If a positive reaction is obtained, the zone in which it occurs should not be used again; testing is discontinued for 24 hours, and then resumed on other injected areas. With each allergen employed, a control test is done in an untreated skin area of the recipient. When a stronger reaction is obtained in the sensitized zone than in the control zone, the result is considered as positive.

Passive transfer tests offer a definite advantage over the direct test methods in such situations as: (*1*) diffuse skin conditions in which normal skin is not available for direct testing; (*2*) when direct testing becomes an ordeal for patient, parent, and physician (12); (*3*) severe asthmatic attacks which do not permit direct diagnostic procedures; (*4*) when results obtained in direct testing are doubtful or contradict the severity of the clinical manifestations.

For Other Forms of Atopy. The same technic as for atopic dermatitis is used for such forms of atopy as asthma, urticaria, or hay fever. The allergens should not be selected according to a rigid scheme, since there is apparently a variation with age in allergic states of different etiologies. Thus, in children up to the age of 2 years food sensitivity is the most frequent cause of allergic states, and the offender can therefore usually be identified from among the limited number of substances listed on page 327. In the 4 year old child the incidence of allergy to food and to inhalants is about equal. By the time the age of 8 years is reached, 85 of 100 allergic children may be expected to be sensitive to environmental substances, inhalants, and contactans. These facts, together with the patient's history, should help the pediatrician to limit, as far as possible, the number of allergens to be used in the initial test series.

Clinical manifestations of hay fever, whatever the patient's age, call for an initial testing with pollen extracts only.

For Dermatomycoses. Immunologically considered, tests for dermatomycoses are tests for hypersensitivity to infection, and they are discussed in the appropriate place (page 339).

INTERPRETATION

As already stated, positive reactions to tests for allergic hypersensitiveness only suggest that the allergens in question may be the etiologic factors in the disease. Negative results, however, do not preclude an allergic etiology.

A positive reaction to patch tests is rather strong support for the diagnosis of contact type dermatitis.

A positive response to egg white, as well as to other allergens, in scratch tests indicates that atopy is most probably present. A negative response to egg white in the scratch test, and to milk, feathers, and dust in intracutaneous tests speaks strongly against the atopic character of the condition.

A negative response to milk in the scratch test has little significance in infants, since sensitivity to milk usually becomes apparent only with intradermal tests.

Hypersensitivity to egg white may also occur as a result of repeated injections of influenza virus (14), since virus vaccines, such as influenza, typhus, and yellow fever, are prepared from the extraembryonic fluid of hen's eggs.

TESTS FOR SERUM HYPERSENSITIVITY

Sensitivity to other than human serum is an allergic manifestation. Its presence can be demonstrated by a positive skin reaction to intradermal injection of small amounts of the serum to which an allergic state exists. The reaction is mainly of the immediate type, but delayed reactions and systemic symptoms may also occur. The test makes it possible to discover individuals who are potential victims of serum reactions. The test material is the serum or antiserum which is about to be used for prophylaxis or treatment.

PEDIATRIC CONSIDERATIONS

The importance of this test in routine pediatric practice cannot be overestimated. Hypersensitiveness to serum may be expected in children who have previously received serum. This is known as artificial sensitization. The group includes all who have been immunized by means of toxin-antitoxin, for example, against diphtheria, or who have been treated with larger doses of serum. There is no doubt, however, that severe serum allergy may also occur in children who have never received any serum or serum derivative but human. This is known as spontaneous or natural sensitization. Children known to be allergic to food, or bacterial environmental allergens, may or may not be sensitive to a specific serum. Ratner (15) points out that the highest incidence of profound or lethal reactions to serum is among spontaneously sensitized "horse asthmatics," being even higher than among individuals with hypersensitivity acquired as a result of previous treatment with large doses of serum. For this reason, even the most carefully taken history can neither reveal nor rule out the possibility of serum allergy as reliably as does the skin test.

Hypersensitivity to human serum is so uncommon as to be negligible.

PROCEDURE

A 1:100 dilution of the serum to be administered is prepared with pysiologic saline, and 0.02 to 0.05 cc. is injected intradermally into the skin of the volar surface of the forearm. If the history of the subject reveals any allergy, a dilution of 1:1,000, or even 1:10,000, should be used. A skin reaction generally appears, if at all, within 30 minutes in the area around the site of injection.

A positive reaction shows varying degrees of erythema, infiltration, edema, or wheal formation. The area covered by these phenomena varies from 0.5 cm. to the entire forearm.

Not infrequently, a delayed skin reaction, identical with that of the immediate reaction, occurs 6 to 24 hours after injection. It may last in varying intensity for more than a week.

Generalized or systemic reactions, which may have a fatal outcome, consist of generalized urticaria, an asthmatic type of dyspnea (bronchiolar spasm), severe chills, and signs of shock. Every degree of these reactions may occur within the first 30 minutes. It is noteworthy that systemic reactions may take place with negative as well as with positive local skin responses.

INTERPRETATION

Ratner's summary (15) of the diagnostic significance of the various types of reaction is given in Table 54B.

TABLE 54B
Significance of Reactions to Intracutaneous Test for Serum Hypersensitivity

Type of reaction	Time of reaction	Significance
Local.	Immediate	Potential hypersensitivity
Local.	Delayed	Mild hypersensitivity
Local + systemic	Immediate	High hypersensitivity
Systemic only. . . .	Immediate	Immediate danger of anaphylactic shock

According to Ratner (15).

Whether or not to administer serum should be decided on the basis not only of the results of skin tests but also on the presence or absence of allergic manifestations in the child's history. The following criteria may be useful in deciding for or against serum therapy: (*1*) If the history and/or skin tests are negative, serum may be used. (*2*) If the history is negative and the skin reaction moderate, caution is advisable. (*3*) If the history is positive and the skin reaction marked, but there is no systemic reaction, serum should be used only if it is mandatory. (*4*) If the history is positive and the test reaction is a generalized urticaria, serum should preferably not be given. In cases in which it is mandatory, tolerance may be obtained by means of certain precautions (see below). (*5*) If the history is positive and the test results in a general reaction involving the respiratory tract, serum should not be given under any circumstances.

When an individual shows hypersensitivity to a certain antiserum, as for instance to horse serum, the response to the corresponding bovine or rabbit antiserum should be tested. If the response to that, too, is positive, but serum must absolutely be used, the risk may be lessened by the following means: (*1*) Injecting first 0.5 cc. of the serum intravenously or subcutaneously, followed by administration of the total amount of serum only if no reaction occurs within 30 minutes. (*2*) Diluting the serum with an equal volume of physiologic saline or 10 per cent dextrose. (*3*) Very slow injection. (*4*) Use of adrenalin and oxygen as soon as signs of shock appear.

Ophthalmic Test. Hypersensitivity to serum may also be ascertained by means of an ophthalmic test (15). As a rule, this test does not give positive reactions as frequently as does the skin test; but a positive ophthalmic reaction indicates more precisely the potential risk of serum therapy. The test may therefore be regarded as a supplement to, rather than a substitute for, the skin test. It is particularly valuable when the reactions to the skin test are equivocal. To use the conjunctiva instead of the skin as test organ for allergic reactions was suggested by Peshkin (16a,b).

For the test a drop of a 1:100 dilution of serum in physiologic saline is instilled in the conjunctival sac of one eye. Varying degrees of reddening and edema of the conjunctiva, burning, itching, and edema, becoming evident within 10 to 15 minutes and usually subsiding within 30 to 60 minutes, constitute a positive reaction. Since violent reactions are possible, a 1:100 solution of adrenalin should be kept in readiness for local use. The test is of little value in children who cry, since the serum is washed away by the tears. Severe reactions, with potential danger to the eye, are rare, and do not lessen the usefulness of the test.

A positive reaction to the ophthalmic test is a definite indication of a high degree of hypersensitivity. Serum must therefore be administered with every precaution. If the history is positive as well, serum should not be given under any circumstances.

If there is no reaction to the ophthalmic test, serum therapy is considered safe, even when the skin test produces marked or very strong local reactions.

B. Allergy to Living Infectious Agents

BRUCELLERGIN SKIN TEST

Infection with any one of the Brucella species results in hypersensitivity to derivatives of the brucella organisms. The sensitivity is tested (17a,b) by intradermal injection of 0.1 cc. of a 1:2,000 dilution of brucellergin, a suspensoid of the brucellar nucleoprotein. Erythema and/or edema at the site of injection, appearing 48 hours after injection, are considered signs of a positive reaction.

A positive reaction indicates past or present infection with one of the three species of Brucella: Br. melitensis, Br. abortus, or Br. suis. A negative result rules out the presence of brucellosis (Malta fever or undulant fever), except in the very early stage of the disease.

TUBERCULIN SKIN TESTS

The primary tuberculous infection produces a tuberculous allergy, i.e., sensitivity to the protein of the tubercle bacillus. Testing with tuberculin is a means of verifying tuberculous allergy clinically. If properly performed, tuberculin skin tests are a safe diagnostic method; the local skin reaction is the only manifestation ordinarily elicited, and focal and systemic reactions, with their potential dangers, are reduced to a minimum.

The skin reaction to tuberculin is of the delayed or bacterial type, appearing gradually 24 to 48 hours after the tuberculin is introduced through the skin by any one of several procedures. These fall into 3 groups: (*1*) cutaneous, in which tuberculin is applied on a superficial and artificial skin lesion; (*2*) percutaneous, in which tuberculin is brought into prolonged contact with a small area of normal, undamaged skin; and (*3*) intracutaneous, in which tuberculin is injected into the superficial layers of the skin.

Three different test methods, one from each of the above groups, will be described here. The reliability of all of them, for individual and group testing, has been assured.

PEDIATRIC CONSIDERATIONS

The best tuberculin test is the one that attains the highest reliability with a minimum amount of tuberculin. In this respect, the Mantoux test is superior to all other tuberculin tests; it permits exact dosage and is most sensitive. The results of the patch test correspond approximately to those of the Mantoux test with 0.01 mg. tuberculin. The Pirquet test is the least sensitive one, failing to elicit a positive reaction in 20 to 30 per cent of positive reactors to the other two tests. Its advantage is the much smaller risk of producing undesirably strong reactions. The ideal methods for mass surveys are the Pirquet test preferably, or the patch test in second place. For a differential diagnosis, however, the result of the intracutaneous (Mantoux) test should be the ultimate criterion.

Before any testing is done, the child's possible hypersensitivity to tuberculin should be considered. Hyperergy may be expected in children suspected of tuberculosis of the skin (erythema nodosum), joints, glands, peritoneum, and pleura. Children of the lymphatic or exudative type, for example, those with eczema, show a constitutional hyperergy. Finally, any child known to be a positive reactor to tuberculin must be considered as potentially hypersensitive when tested anew. In all these conditions, therefore, only such tests as are not very sensitive should be used, in order to avoid undesirable reactions. On the basis of available data, the following procedure (18) seems advisable:

(*1*) For mass surveys (case finding), a single application of the Pirquet test or of a patch test.

(*2*) For diagnostic testing of an individual child without known hypersensitivity to tuberculin, the initial test may be the Pirquet test, the patch test, or the Mantoux test, with 0.01 mg. of old tuberculin (OT), or 0.00002 mg. of P.P.D. tuberculin. If the initial test is negative, the Mantoux test should be performed 2 to 4 days later, using 0.1 mg. OT or 0.005 mg. P.P.D. If the second test is negative, another Mantoux test is done 2 to 4 days later, using 1 mg. OT. If the reaction is still negative, the child may safely be considered nonallergic to tuberculin.

(*3*) For diagnostic testing of a child presumably hypersensitive, the Pirquet test is done first; it is followed for the second testing by the patch or Mantoux tests, using 0.01 mg. OT or 0.00002 mg. P.P.D. for the latter test, and by the Mantoux test for the third testing, using 0.1 mg. OT or 0.005 mg. P.P.D.

Tuberculin tests should be made during afebrile periods. When a negative reaction is obtained during, or shortly following, the acute stage of an illness, the test should be repeated when convalescence is complete. The dose used, however, should be the same as the initial one, not an increased dose. Frequent repetition of tuberculin tests in positive reactors is useless and may even be harmful. Infants exposed to open pulmonary tuberculosis should be retested every 6 months up to their third year, but only so long as they react negatively.

PROCEDURES

Preferably, the tuberculin to be used for all tests is the Old Tuberculin Koch (OT), as standardized by the Hygiene Committee of the League of Nations in 1928.

Another preparation more recently recommended is the purified protein derivative (P.P.D.), developed by Seibert (19); it should be used only for intracutaneous testing.

Cutaneous Tuberculin Test (Pirquet Test) (20). The inner flexor surface of the forearm is cleansed with ether or benzine. After a few minutes, 1 drop of OT is applied with a pipet on each of 2 spots, about 10 cm. apart, of the cleansed skin area. Using the Pirquet needle (20), the scarifier devised for scratch tests (9), or any other needle, 3 scratches are made—one midway between the 2 drops of tuberculin to serve as a control, the other 2 through the tuberculin drops themselves. The outer layers of the skin only should be abraded, and no blood should be drawn. After 5 minutes the tuberclin remaining on the skin is removed with cotton. After 24 to 48 hours the control scratch should show a scurf about 1 mm. in diameter and of brownish discoloration.

The appearance of various degrees of inflammation (redness, edema, necrosis) after 48 or more hours around one or both of the tuberculin scratches is interpreted as a positive reaction. Reactions lasting less than 48 hours are of traumatic or nonspecific origin, and have the same significance as negative reactions.

Tuberculin Patch Test (Percutaneous Test). In this test tuberculin is applied to the surface of the skin, instead of into or under the skin, by means of an adsorbent material or patch. Paper towels, gauze, cotton, blotting paper, adhesive plaster, filter paper, ointment, and Fuller's earth have all been used as adsorbents. In

Europe, tuberculin ointments have been particularly favored. The patches can be prepared and attached to adhesive plaster in the laboratory or the physician's office. A commercial preparation of patches is also available. In recent years the use of patch tests has become widespread.

Patch Test with Noncommercial Preparation. The method described is that of Grozin and Reisman (21). A piece of paper towel, gauze, cotton, blotting paper, or filter paper is placed on a dish or other smooth surface, moistened with OT, and allowed to dry. The treated material is then cut into 1 cm. squares, forceps being used in handling the material. A 2 cm. wide strip of adhesive plaster is spread on a table, the squares are placed on the adhesive at intervals of about 5 cm., and the strip is cut into pieces, each containing a tuberculinized square in the center. The pieces of adhesive are laid out on a smooth surface, such as a dish, with the adhesive surface downward, and are stored until needed. For the test, one of these pieces is placed on the back, chest, or forearm, after the spot has been cleansed with ether or acetone. The patch is removed after 48 hours, and results are read after an additional 48 hours.

Edema, papules, vesicles, and redness, usually limited to the surface in contact with the tuberculin, characterize the positive reaction.

Patch Test with a Commercial Preparation. The method described is that of Vollmer and Goldberger (22). The patch consists of filter paper impregnated with OT (2 squares) and a broth control (1 square) held in place by adhesive plaster.

The patch is preferably applied in the intrascapular region, although the inner aspect of the forearm or the anterior chest wall may be used. When hair is present, it should be shaved off. The site is cleansed with ether or acetone, and allowed to dry. The adhesive is then applied; the palm of the hand is held against the tape for a few seconds to warm the adhesive, and the area massaged well for at least 10 strokes. After 48 hours, the adhesive is removed. The results are read after the lapse of another 48 hours.

A positive reaction is the same as described above.

Intracutaneous Tuberculin Test. In the Mantoux test (23), dilutions of tuberculin are injected intradermally on the flexor side of the forearm, the desired amount of tuberculin being contained in 0.1 cc. of the dilution. A special beveled shoulder needle, of 26 gage and $^3/_{16}$ inch cannula, is recommended, but any small needle of similar size may be used. A special tuberculin syringe facilitates accurate measurement of the required amount of tuberculin.

Syringes used for tuberculin testing should not be used for any other purpose.

The tuberculin injected may be Old Tuberculin (OT), or purified protein derivative (P.P.D.). OT stock solutions of 1:100 and 1:1,000 are obtained by diluting the commercial concentrated OT with 0.5 per cent carbolized physiologic saline solution. Stock solutions may be kept in the refrigerator up to a month. Weaker dilutions are prepared from these stock solutions with distilled water as needed.

P.P.D. solutions are obtained by adding 0.5 cc. of buffered diluent to a vial containing a tuberculin tablet of first strength (0.0001 mg. P.P.D.) or second strength (0.025 mg. P.P.D.). Packages containing P.P.D. tablets and diluent are commercially available.

The actual amount of tuberculin injected with each 0.1 cc. of the different dilutions is:

Dilution	Milligrams
1:10,000	0.01 OT
1:1,000	0.1 OT
1:100	1.0 OT
First strength	0.00002 P.P.D.
Second strength	0.005 P.P.D.

Infiltration and hyperemia about the site of the tuberculin injection characterizes the positive reaction. It reaches a maximum in about 48 hours, and disappears in 4 to 8 days. Very strong, undesirable reactions may produce vast areas of inflammation, ulceration, necrosis, or regional lymphadenitis. Pseudoreactions disappear within 36 hours.

INTERPRETATION

In the routine health examination of individuals and in mass surveys, a negative reaction to a single skin test selected as outlined above indicates that in all probability the child has no active tuberculous disease. It does not, however, tell definitely whether an inactive infection is present or not.

A positive reaction proves only that the child has been host to the tubercle bacillus. Whether there are other consequences than the established tuberculous allergy must be shown by clinical examination.

The interpretation of tuberculin tests used, as outlined above, for the diagnostic study of a given clinical condition, is somewhat involved.

Negative reactors may be: (*1*) Children not infected with tuberculosis. (*2*) Infected children who had been tuberculin positive in previous tests but who are now negative. This may be due to (*a*) transient loss of allergy during and shortly after infectious diseases, such as measles or whooping cough; (*b*) immunobiologic healing, i.e., complete restitution to an immunologic condition, as it existed before the tuberculous infection; (*c*) irreversible loss of allergy because of a breakdown in the production of antibodies, such as occurs in the terminal stage of tuberculous meningitis, miliary tuberculosis, or marasmus. (*3*) Infected children who are not yet tuberculin positive, but who will be positive as soon as allergy has developed. Since this "incubation time" of tuberculous allergy may vary from several days to 2 months, retesting will provide a clear answer (24).

Positive reactors under 4 to 5 years of age are likely to be ill from primary tuberculous infection, and may have active tuberculosis. If they are 5 to 6 years or older, they should not be regarded a priori as tuberculous. The majority of positive reactors in this age group have an "infection" only. According to Nelson (25), the term "infection" in this restricted sense is intended to connote a state of inactivity that is either latent or quiescent, or healed.

The incidence of positive tuberculin reactions from quiescent infections increases with age. While infants and young children are apt to contract active tuberculosis from the primary invasion of the tubercle bacilli, the majority of older children escape with a primary lesion which remains quiescent indefinitely. In them, the main consequence is the development of tuberculous allergy, and a questionable immunity. The immediate danger of primary infection becomes greater again during the adolescent period.

In the individual case of a positive reaction to tuberculin, the inactivity or the degree of activity of the tuberculous process must be established definitely by other methods of examination.

TESTS FOR SENSITIVITY TO FUNGUS ALLERGENS

To test for hypersensitiveness to members of the Trichophyton group or to yeastlike fungi, a small amount of allergenic derivatives of the microorganisms is injected intradermally. The reaction is of the delayed type and greatly resembles the tuberculin reaction.

In young children differentiation between fungus infections and eczematous eruptions due to other causes should ultimately be based on dermatologic criteria. But if the need for further evidence is felt, skin tests may well be used. Infections with yeastlike organisms are more common in children than trichophyton infections.

Either trichophytin, prepared from a mixture of Trichophyton fungi, or oidiomycin, extracted from Monilia albicans, is employed as the allergenic test substance. Both are commercially available, and are used in dilutions of 1:50 and 1:100, respectively.

The test (26) consists of the injection of 0.1 cc. of the allergenic substance into the cleansed skin of the forearm. The result is read after 48 hours. A papule, surrounded by erythema or edema, reaching its maximum intensity after 48 hours, is the usual positive reaction. Pseudoreactions may occur; they are of the wheal type, appear earlier, and have faded after 24 to 48 hours. Focal and systemic reactions, such as occur in tuberculin tests, have also been observed.

A positive reaction supports the diagnosis of fungus and yeast infections, respectively. Persons with dermatophytosis almost invariably show a positive skin reaction to trichophytin.

Absence of reaction is even more significant, since it practically rules out fungus infection as an etiologic factor.

Histoplasmin Test. This is an intradermal test designed to detect histoplasmosis. The reaction seems to be nonspecific; there is a high incidence of cross reactions with blastomycosis and other fungus infections (27a). The sensitivity to the antigen prepared from Histoplasma capsulatum may, according to Christie and Peterson (27b), merely be an index of the over-all problem of pulmonary and systemic mycotic infections. It remains to be seen whether the test will serve, like the tuberculin test, as an "exclusion test" in infection with H. capsulatum.

TESTS FOR SENSITIVITY TO ECHINOCOCCUS ANTIGEN

The Casoni test (28) provides a biologic diagnosis of echinococcosis (hydatidosis), a condition caused by the cystic larval (hydatid) stage of Echinococcus granulosus. An individual harboring the parasite develops a specific sensitization by absorption of the hydatid antigen. Such sensitivity can be demonstrated by means of the skin test.

The reaction is not species specific but group specific; antigens prepared from other cestodes, substituted for hydatid antigen, will elicit positive results. The antigens used are (*1*) saline extracts (commercially available) of the common rabbit cestode, Cysticercus pisiformis, or of Taenia taeniaformis, a tapeworm commonly found in cats (29), or (*2*) pooled sterile hydatid fluids obtained from sheep, cattle, or man, which may be had on request to the National Institute of Health.

The test dose is 0.01 to 0.1 cc. of the antigen. It is injected intradermally into the volar surface of the forearm, and a control injection with saline solution should not be omitted.

Positive reactions, consisting of an urticarial wheal with or without pseudopodia, surrounded by a zone of hyperemia, usually appear within 15 to 20 minutes. They fade shortly afterward. In rare cases, the reaction may develop more slowly, reaching its maximum only 24 hours after injection. These slow reactions consist of edema and erythema around the point of injection. Pseudoreactions may occur when hydatid fluid from sheep or cattle is used as antigen, as a result of hypersensitiveness to foreign protein.

Positive reactions support the diagnosis of cestode infection. Even more trustworthy are negative reactions: they rule out the presence of hydatid disease. The intensity of positive reactions probably depends upon the status of the disease. Negative skin reactions have been reported in individuals shown to be harboring calcified echinoccic cysts.

TEST FOR HYPERSENSITIVITY TO TRICHINELLA ANTIGEN

The test (30a,b) represents a means of detecting sensitization to Trichinella spiralis, which causes trichinosis. The antigen used is an extract from powdered Trichinella larvae, available commercially. The test is performed by intradermal injection into the forearm of 0.1 cc. of the antigen, diluted 1:10,000. A control injection is made with 0.1 cc. of buffered saline.

A positive skin response may appear immediately after injection if the individual was infected with trichinosis about 2 to 3 weeks previously. The immediate reaction consists of an urticarial wheal, surrounded by an area of erythema. A slow type of reaction, reaching its maximum within 24 hours, is commonly observed during the first days of the disease, and in the so-called "quiescent" cases of long duration. Here the skin reaction greatly resembles the erythematous–edematous type of the tuberculin reaction.

Negative results are more significant than positive reactions.

AGGLUTINATION TESTS

TEST FOR HETEROPHIL ANTIBODIES

Substances known as heterophil or heterogenetic antibodies occur in the blood, and have a specific action on the erythrocytes of other species. In normal human serum there are heterogenetic agglutinins, lysins, etc., which act on the red cells of various animal species. A marked increase of agglutinins for sheep red cells has been observed in patients suffering from infectious mononucleosis methods (1) that the serum antibodies for sheep red cells found in normal man, in infectious mononucleosis, and in serum sickness are not identical.

PROCEDURES

Macroagglutination Method. The method described is that of Paul and Bunnell (31). From 3 to 5 cc. of blood are withdrawn from the patient by venipuncture; the serum is separated and inactivated by incubation at 56 C. for 15 to 30 minutes. A series of 12 tubes is set up, and 0.5 cc. of physiologic saline solution is transferred into each tube except the first. To each of the first 2 tubes is added 0.5 cc. of the inactivated serum; then 0.5 cc. of the contents of tube 2 is transferred into tube 3, and so on successively to tube 11, from which 0.5 cc. is discarded. Tube 12 serves as control. Then 0.5 cc. of a 2 per cent suspension of washed sheep red corpuscles in saline is added to each tube; the red cells should be at least 1 day old. The final dilutions of the series range from 1:2 to 1:2,048.

The set of tubes is incubated in a water bath at 37 C. for 1 hour, and then placed in the refrigerator overnight. The next morning each tube is inverted 3 times, and examined for agglutination.

According to Hollander (32), instead of incubating the mixtures, they may be centrifuged for 5 minutes at about 1,000 r.p.m., and then shaken and read immediately for agglutination.

Microagglutination Method. The method described is that of Butt and Foord (33). One loopful of blood serum to be tested and 4 loopfuls of a 2 per cent sheep red blood corpuscle suspension in saline are mixed in a hanging drop preparation. If agglutination occurs, it can be observed almost immediately.

INTERPRETATION

In the macromethod an agglutination titer up to 64 is considered normal. Agglutination occurring in final dilutions of 1:128 suggest infectious mononucelosis or serum sickness, while agglutination in final dilutions of 1:256 or more is pathognomonic for these diseases.

In the micromethod, if agglutination occurs at all, it is a sign of infectious mononucleosis or serum sickness. Serums showing agglutination in dilutions below 1:256 when examined by the Paul-Bunnell method are negative when examined by the micromethod.

Recent observations have thrown doubt on the specificity of the Paul-Bunnell test. A positive titer above the so-called upper border of normal may appear in an appreciable number of children with acute infections of the upper respiratory tract (34); and negative reactions may occur in children with infectious mononucleosis, due to the so-called blocking effect (35).

TESTS FOR Rh FACTOR AND Rh AGGLUTININ

The problem of the rhesus antigen in medicine, and its serologic and genetic ramifications, have become the domain of specialists, but the basic concepts of the subject and its practical implications are of concern to every clinician. It is only such limited information that can be conveyed to the reader in the discussion which follows. Detailed information may be found in the original publications (36–41), and in Wiener's most recent review of the subject (42).

Blood Groups and Rh Factor. The four classic blood groups in man are characterized by the presence or absence of one or both of the group-specific antigenic substances (A, B) in the red cells, and of the isoagglutinins (anti-A, anti-B) in the serum. Under normal conditions, correspondent antigens and agglutinins, for example, A and anti-A, never occur in the same blood, since the inevitable result of such occurrence is agglutination of the red cells.

A number of other agglutinogens have been identified in human red cells, leading to the establishment of "subgroups." Of these, the M, N, and Rh factors are of clinical importance. The chief characteristic of these new types is the absence of antibodies for them in human serum; anti-M, anti-N, and anti-Rh isoagglutinins practically never occur in *normal* human serum.

The presence of the Rh factor in human red cells was first discovered by Landsteiner and Wiener (36) when samples of human erythrocytes which they had mixed with antirhesus serums showed agglutination. The antirhesus serums were obtained from rabbits which had previously been injected with the blood of rhesus monkeys; hence, the factor was designated "Rh." The Rh factor has been found to be present in the erythrocytes of about 85 per cent of the white population.

Subtypes of Rh Factor and Rh Agglutinin. The Rh factor exists in a number of subtypes, probably inherited as a series of allelic genes. In addition to the original rhesus factor, Rh_0, detected by animal antirhesus serum, two other Rh factors, rh′ and rh″, have been identified by the use of human serums containing anti-rh′ and anti-rh″. Wiener (43,44) characterizes and designates the properties of the human Rh agglutinogens in the erythrocytes and of the Rh agglutinins in the serum as follows:

> The Rh factors Rh_0, rh′, and rh″, in combination, give rise to five agglutinogens, namely, Rh_0, rh′, rh″, Rh_1 (short for Rh_0rh′), and Rh_2 (short for Rh_0rh″).
>
> Rh_1 is also designated as Rh_0′, and Rh_2 as Rh_0″. A fourth and rarer Rh factor, rh‴ (c^w), has recently been found by Callender and Race (44a).
>
> The anti-Rh agglutinins of human serum are three in number, corresponding to the three Rh factors—anti-Rh_0, anti-rh′, and anti-rh″. Human serum may contain one or two of the Rh agglutinins. Thus, there are five common varieties of human Rh antiserum: anti-Rh_0, anti-rh′, anti-rh″, anti-Rh_1 (containing anti-Rh_0 and anti-rh′), and anti-Rh_2 (containing anti-Rh_0 and anti-rh″).
>
> The animal antirhesus agglutinins are all of the same specificity, namely, anti-Rh_0. Animal antirhesus serum and human anti-Rh_0 serum are known as standard anti-Rh serum.
>
> The blood factors designated as Hr are related to Rh in that they occupy the place of Rh in Rh-negative cells. Corresponding to

the three varieties of Rh there are probably three different Hr antigens (42).

Table 54C gives the classification of the Rh blood types. Almost all clinical problems caused by the Rh factors involve factor Rh$_0$. Wiener (42) states: "Ordinarily, therefore, when the terms "Rh positive," "Rh negative," "Rh factor," "anti-Rh agglutinin" are used without special qualification, it is the original rhesus factor or Rh$_0$ factor that is referred to, and not rh′ or rh″."

TABLE 54C

Classification of Rh Blood Types

	Bloods lacking Rh$_0$				Bloods containing Rh$_0$		
	Reactions with antisera				Reactions with antisera		
Type	rh′	rh″	Rh$_0$	Type	rh′	rh″	Rh$_0$
rh....	–	–	–	Rh$_0$......	–	–	+
rh′...	+	–	–	Rh$_1$(Rh$_0$′).	+	–	+
rh″...	–	+	–	Rh$_2$(Rh$_0$″).	–	+	+
rh′rh″.	+	+	–	Rh$_1$Rh$_2$	+	+	+

From Wiener (45).

"Rh testing" refers to the procedure by which a blood sample is examined for agglutination by a standard anti-Rh serum alone, to reveal the presence or absence of the Rh$_0$ factor. The examination of a blood sample with the aid of human serums anti-Rh$_0$, anti-rh′, and anti-rh″ is termed "Rh typing." By this procedure an individual can be classified within one of the eight Rh types.

Isoimmunization to Rh Antigens. In contrast to the M and N substances, the Rh factor calls forth considerable antigenic activity in man. Thus, a person with Rh-negative blood may develop Rh antibodies in the serum if he receives one or more transfusions of Rh-positive blood, and the presence of these antibodies in sufficiently high titers results in intragroup hemolytic reactions upon subsequent transfusions of Rh-positive blood. Isoimmunization may also occur during pregnancy, if the woman is Rh negative and the fetus, as the result of an Rh-positive father, is Rh positive. The mother's constant exposure to the child's Rh antigen in such cases may result in sensitivity to the Rh factor, i.e., in the development of anti-Rh antibodies in the mother's serum. These antibodies are introduced into the fetus by way of the circulation and exert a harmful effect on its Rh-positive cells, possibly resulting in hemolytic disease of the newborn. In such erythroblastotic infants the paradox exists, as Wiener (41) puts it, of an antigen (Rh) being present in the body simultaneously with its specific antibody. Fortunately, only 1 in 25 to 50 negative women exposed to the Rh antigen through pregnancy becomes sensitized (42). On the other hand, if Rh-negative persons are injected with Rh-positive blood, as many as 1 out of 2 becomes sensitized.

Indications for Rh Tests. The clinical importance of such tests lies in the fact that isoimmunization against the Rh antigens has been recognized as a cause of intragroup hemolytic reactions after transfusion and of hemolytic disease of the newborn (erythroblastosis foetalis). Both conditions are of particular concern to the pediatrician.

Rh testing is now customarily performed on both husband and wife when the latter becomes pregnant. If the test results indicate that maternal sensitization may be expected, the mother's serum must be tested for Rh antibodies at regular intervals, particularly toward the end of pregnancy. Isoimmunization can then be detected at the onset, and an attempt be made to deliver the infant before he has suffered serious damage.

If born alive, the infant is immediately tested for the Rh factor. Only human anti-Rh serums should be used for these tests, since many animal antirhesus serums agglutinate Rh-positive as well as Rh-negative blood of newborn infants. The maternal Rh antibodies responsible for the hemolytic disease have also been demonstrated in the infant's blood.

Apart from the neonatal period, Rh testing in children is also advisable prior to transfusions. Both donor and recipient must be tested for Rh compatibility, in order to avoid an intragroup hemolytic reaction. While no longer ago than 1945 Wiener (41) stated that there was no need for Rh testing before every transfusion if certain rules were observed, such testing is now routinely done prior to transfusion, not only to avoid reactions but also to avoid sensitization, particularly in women. If the child to be transfused is found to be Rh negative, only compatible Rh-negative blood should be used for the transfusion. If such blood is not available, washed red cells of the mother's citrated blood may be used.

These precautions would prove beneficial, with respect to future transfusions, even in children in whom Rh testing might be disregarded because they had not been previously transfused.

"Exchange transfusions" are the best means of treating severe forms of erythroblastosis foetalis (44b–e).

Methods of Testing. The Rh properties can be determined by agglutination tests. To identify a person as Rh positive or Rh negative, a suspension of his red cells is mixed with standard anti-Rh serum (page 348). If the red cells agglutinate, the individual is Rh positive; if they do not agglutinate, the person is Rh negative. Similarly, examination of serums for anti-Rh agglutinins is done by mixing the unknown serum with red cells known to be Rh positive. Agglutination proves that the serum contains anti-Rh agglutinins, and vice versa.

With the rapidly growing knowledge of the Rh antigen, the technic of these test procedures has also been undergoing rapid change. The discovery (*1*) of the existence of the aforementioned Rh subtypes, and (*2*) of the presence of Rh antibodies in some Rh antiserums which are distinctly different from anti-Rh agglutinins is mainly responsible for the increased complexity of Rh tests. While in routine practice the new and very complicated test methods which have been evolved as a result of these discoveries can be dispensed with, the second of the two discoveries has important implications for the technic of clinical Rh tests.

The serum of persons sensitized to the Rh factor may contain, in addition to Rh agglutinins, another variety of anti-Rh antibodies termed "blocking antibodies" or "glutinins" (univalent Rh antibodies) (46). They do not cause agglutination, but by combining with Rh positive cells they block the agglutinating action of the anti-Rh agglutinins, whether these agglutinins are present in the serum to be tested or are subsequently added to the cells with a potent anti-Rh serum.

When this fact was established, it became obvious that the classic agglutination tests would be inadequate to detect Rh sensitization in a number of persons who are actually sensitized, or to detect all cases of Rh incompatibility. To avoid these pitfalls, Wiener has adapted the technic and methods of Rh tests to meet the requirements of the new concept, in either of two ways. The old test procedures may be supplemented by the new "blocking test," or the principle of the new test may be incorporated into the original procedures themselves. The innovation then consists in the use of normal plasma instead of normal saline solution as the diluent for the red cell suspension. The patient's own serum, group AB serum, or any other compatible plasma, or human or bovine serum albumin have proved effective in overcoming the "blocking" action. Thus, for example, the "conglutination test" is the new form of the tube incubation test for Rh sensitivity, making use of serum instead of saline.

To facilitate the rational use of the various tests to be described, and for a better understanding of the specific arrangement of each of them, the tests are listed here according to the purpose for which they are used.

(*1*) Identification of Rh-positive and Rh-negative persons (Rh testing):
  (*a*) Tube incubation test.
  (*b*) Modified tube incubation test.
  (*c*) Slide test.

(*2*) Detection of group A/B and intragroup (Rh) incompatibility (compatibility tests):
  (*a*) Modified tube incubation test (cross-matching).
  (*b*) Modified slide test (cross-matching).

(*3*) Detection of Rh sensitivity (detection of Rh antibodies):
  (*a*) Combined tube incubation and blocking tests.
  (*b*) Conglutination test.
  (*c*) Slide test.

Procedures

The use of weak serums in Rh studies led to many difficulties and to serious mistakes. For reasons already given (page 345), human antiserums are preferable to animal antirhesus serums. Human serums are obtained from Rh negative mothers who have had erythroblastotic infants or from Rh-negative persons sensitized to the Rh antigen by repeated transfusions of Rh-positive blood.

A satisfactory antiserum must fulfill the following requirements: (*1*) Its specificity must be known (page 348). (*2*) Its potency (titer) must have been ascertained. (*3*) It must be free of active normal isoantibodies (anti-A, anti-B, or both); the inhibition of these antibodies, if they are present, can be accomplished by adding boiled saliva of secretors of group A or B, by absorption with pooled A_1rh and Brh cells, or by using the Witebsky A and B substances in solution (47a). Satisfactory anti-Rh serums of known specificity are available through commercial channels, from local blood banks, or from blood donor services.

Anti-Rh typing serums also may be labeled according to the terminology proposed by Fisher and Race (47b). Both the Wiener and the Fisher-Race system will soon be found on labels of antiserums supplied in the United States (47c). A comparison of the nomenclature of the individual antiserums of the two systems follows:

Wiener	Fisher-Race
Anti-rh′	Anti-C
Anti-Rh$_0$	Anti-D
Anti-rh″	Anti-E
Anti-hr′	Anti-c
Anti-Hr$_0$	Anti-d
Anti-hr″	Anti-e
Anti-Rh$_1$ (anti-Rh$_0$′)	Anti-C+D
Anti-Rh$_2$ (anti-Rh$_0$″)	Anti-D+E

Rh TESTING

These procedures serve to identify an individual as Rh positive or Rh negative, and are carried out in addition to the classic blood grouping. Two varieties of serums are now available for Rh testing: serums containing agglutinins (standard Rh antiserums) and those containing univalent or blocking antibodies (conglutinating serums). The technic of Rh testing varies depending upon the type of testing serum available. With an agglutinating serum, the tube incubation test is employed; for conglutinating serums, the modified tube incubation technic or the slide must be used. It is therefore essential to note the designation on the label of the serum procured for testing. Reagents needed for either of these tests are:

(*1*) Potent anti-Rh serum (agglutinating or conglutinating).
(*2*) Known Rh$_0$ positive red cells.
(*3*) Known Rh$_0$ negative red cells.

Tube Incubation Technic. Venous blood obtained by venipuncture is preferable to capillary finger blood. Small samples from the finger, mixed with tissue juices, and hemolyzed samples give poor results (48). Clotted blood, or whole blood mixed with dried oxalate powder as anticoagulant, may be employed; if the former is used, the stoppered tube containing the clotted blood is shaken vigorously to free as many cells as possible from the clot and then allowed to stand until all the coarse particles have settled. For the test, the supernatant suspension of red cells in serum, while still fresh, or the whole oxalated blood specimen is washed once by centrifuging, the supernatant is discarded, and the red cells are resuspended in enough saline solution to make a 2 per cent suspension in terms of blood sediment.

One drop of this suspension is mixed with one drop of agglutinating anti-Rh serum in a 10 × 75 mm. test tube. For the controls, known Rh-positive and Rh-negative red cell suspensions, similarly prepared, are mixed with the test serum. The tubes are incubated at 37 C. for 1 hour, after which they are examined both macroscopically and microscopically for agglutination. When the result is doubtful, "the reactions can often be improved by centrifuging the tubes for 1 minute at low speed (500 r.p.m.), or by allowing the mixture to stand another hour or two at room temperature" (Wiener).

It is a peculiarity of the agglutination reactions due to anti-Rh serum that the clumping is frequently more fragile than in agglutination reactions produced by ordinary grouping serums. Agglutination is a sign of the presence of the Rh factor; bloods not showing agglutination are Rh negative.

Modified Tube Incubation Technic. For univalent or conglutinating testing serums, the following procedure has been recommended by Diamond and Denton (49). Instead of using a 2 per cent suspension of red cells in saline the test is carried out with erythrocytes washed twice with saline and suspended in a 20 per cent solution of bovine albumin to make a 2 per cent suspension in terms of blood sediment. The 20 per cent albumin solution is prepared by diluting a commercially available 30 per cent bovine albumin solution with saline. 2 drops of this suspension are mixed in a test tube with 1 drop of the univalent anti-Rh serum. The incubation time is reduced to 5 to 10 minutes. The control is treated similarly. In other respects, the procedure is similar to that of the tube incubation test, described above.

Slide Technic. Oxalated whole blood or clotted blood (see page 348) may be used. One drop of univalent testing serum (conglutinating serum) is placed on an open slide and 2 drops of the blood are added. Serum and blood are thoroughly mixed with an applicator and spread over an area about 1 inch in diameter. The slide is then placed on a warm stage and tilted back and forth slowly. Readings are made with the naked eye. Reactions usually appear in 30 seconds and are complete within 2 minutes when oxalated blood is employed. With samples from clotted blood, it takes 2 to 3 minutes to form the coarse, dark red granules which characterize a positive conglutination. If the blood is Rh negative, the mixture will remain homogeneous. Controls are run with known Rh-positive and Rh-negative cells.

Pseudo-agglutination (rouleaux formation) which may cause doubtful test results can be eliminated by the addition of one drop of saline solution after the reaction has had time to develop, i.e., not until the slide has been observed for at least 5 minutes. Doubtful or negative results, particularly when obtained with blood from an infant or from umbilical cord blood, call for the tube incubation test, using agglutinating serum.

DIRECT MATCHING TESTS (MODIFIED COMPATIBILITY TESTS)

Undiluted samples of venous whole blood are used, prepared by the addition of dried oxalate or from a clotted specimen, as described on page 348. Saline must not be used. These whole blood samples represent suspensions of red cells in their own undiluted serum.

Tube Incubation Technic. A small volume, for example, 0.5 cc., of the recipient's blood is added to an equal volume of the donor's serum in a 10 × 75 mm. test tube. The same amounts of the donor's blood and of the recipient's serum are mixed in another tube. Both tubes are shaken gently, and incubated at 37 C. for 1 hour. The sedimented cells are dislodged by gentle shaking, and a few drops of the suspension are transferred onto a slide by means of a glass rod, and examined microscopically (low magnification) for agglutination.

If there is agglutination, the prospective donor's blood is incompatible with the recipient's blood.

To rule out autoagglutination, a control is run, using a mixture of the patient's serum and his own blood cells.

Slide Technic (50). Using whole blood–red cell suspensions, as in the preceding technic, 0.2 to 0.5 cc. of the donor's blood suspension is mixed with the same amount of the recipient's serum on the open slide, which is then placed on a warm stage or moderately warmed over a 25 watt bulb. The slide is tilted back and forth slowly, and after an interval of a few seconds to 3 minutes the mixture is examined both macroscopically and microscopically for agglutination. This must be done before the mixture dries. The same procedure is carried out with similar amounts of the donor's red cell suspension and the recipient's serum.

If the mixture on both slides has remained homogeneous, the bloods of donor and recipient are compatible. If there is agglutination on one or both slides, the bloods are incompatible.

Pseudoagglutination causes rouleaux formation of the red cells; it must be clearly distinguished from true agglutination. If there is any doubt about the diagnosis, addition of 1 drop of saline will eliminate the pseudoagglutination. The saline will not interfere with the mechanism of the reaction, provided it is added after the reaction has fully developed.

If capillary blood has to be used for the test, a drop of the donor's serum is placed on an open slide, several crystals of sodium or potassium oxalate are added, and the mixture is stirred with a glass rod until all the crystals have dissolved. The first drops of blood from the punctured finger of the recipient are wiped off; 2 drops are then gently squeezed onto the slide and mixed with the serum by means of a glass rod. In similar fashion, the recipient's serum is treated with oxalate and mixed with finger blood from the donor. The remainder of the procedure is the same as above.

TESTS FOR Rh SENSITIZATION

The presence of Rh antibodies in the serum of persons who have acquired sensitivity to the Rh factor may be demonstrated by one of several methods. These are: (*1*) agglutination test; (*2*) test for blocking antibodies; (*3*) conglutination test; (*4*) open slide test.

Agglutination Test (51).

Reagents.

(*1*) Rh-positive, group O blood cells. If bloods known to be Rh_1 and Rh_2 are available, 2 different bloods of each type are used, i.e., 4 Rh-positive samples; if the specificity of the available Rh-positive bloods is not known, it is advisable to use 5 to 10 different bloods.

(*2*) Rh-negative, group O red cells.

Each variety of the cells to be used is suspended in saline solution so that the concentration is approximately 2 per cent in terms of blood sediment (page 349).

Technic. The serum to be tested is separated from the clot and inactivated at 56 C. for 15 minutes. A drop of each of the Rh-positive and Rh-negative red cell suspensions is transferred into separate 10 × 75 mm. test tubes, and 1 drop of the serum is added to each of the tubes. The mixtures are incubated for 1 hour at 37 C., and then centrifuged for 1 minute at 500 r.p.m. The sediments are gently dislodged by twisting the tubes, and the mixtures are examined for the presence or absence of agglutination. Its presence in all or most of the Rh-positive cell mixtures indicates that the serum belongs to a person sensitized to the Rh antigen.

If agglutination fails to develop, the test for blocking antibodies must be carried out.

By preparing and testing progressively doubled saline dilutions of the serum, its potency or titer of agglutinins can be determined quantitatively. In the same way, titrations of the blocking antibodies can be carried out by the conglutination technic.

Blocking Test (46). This test is carried out only on serums that have given negative results with the agglutination test above.

Reagents.

(*1*) Potent anti-Rh agglutinating human serum (standard anti-Rh serum).

(*2*) Group O, Rh-positive red cells, as in the preceding test, suspended in saline solution so that the concentration is 2 per cent in terms of blood sediment.

Technic. A drop of a 2 per cent suspension of Rh-positive cells and 1 drop of the patient's serum are mixed in a small test tube and allowed to interact in a water bath at 38 C. for 30 to 60 minutes. Then a drop of anti-Rh serum is added, and the reaction is read after an additional incubation period of 30 to 60 minutes. A control is run with saline instead of the unknown serum. If blocking antibodies are present, no agglutination will occur, or the clumping will be distinctly weakened.

Agglutination confirms the absence of Rh antibodies in the serum tested, and indicates that the person is not sensitized to the Rh antigen.

The combined use of the agglutination and blocking tests is a laborious procedure. Rh sensitization can be more conveniently ascertained by Wiener's more simple conglutination test or Diamond and Abelson's slide test.

Conglutination Test (42,52). According to Wiener, the test always gives positive reactions "when the patient's serum contains Rh agglutinins or Rh blocking antibodies, or both, and occasionally when the other two tests [agglutination and blocking tests] are negative or inconclusive." The technic is similar to that of the agglutination test, except that the use of saline solution is carefully avoided.

Reagents.

(*1*) Group O, Rh-positive cells, preferably 2 separate bloods of types Rh_1 and Rh_2.

(*2*) Rh-negative, group O cells.

The red cells are suspended in their own plasma or serum (page 350), in inactivated group AB serum, or in 20 per cent solution of bovine albumin, the concentration of the blood cells being 2 per cent in terms of blood sediment (page 349).

Technic. A drop of each of the Rh-positive red cell-serum suspensions is transferred into separate 10 × 75 mm. test tubes, and 1 drop of the patient's serum is added to each tube. A control is run with an Rh-negative cell suspension. All the tubes are incubated for 1 hour at 37 C. The sediment is then dislodged by twisting the tube gently, and the mixtures are examined for clumping, or "conglutination," as it is termed in this test. A detailed explanation of his concept of the conglutination reaction may be found in Wiener's recent monograph on the Rhesus antigen (42).

A clumping reaction is the most reliable indication of the presence of Rh antibodies in the serum.

Open Slide Test. This simple procedure is recommended by Diamond and Abelson (49,53). It is as reliable as the tube conglutination test, and may be based on the same serologic reaction.

Fresh, whole, oxalated, group O, Rh-positive blood, of normal hematocrit value, is mixed with an equal amount (approximately 0.2 cc.) of the patient's serum on the open slide. The slide is moderately warmed on a stage over a 25 watt bulb, and gently tilted from time to time. A control with Rh negative blood is run at the same time.

If the test is positive, readily discernible clumping appears after an interval ranging from a few seconds to 3 minutes or longer, depending on the antibody titer.

A positive reaction is a sign that anti-Rh antibodies are present in the patient's serum, i.e., Rh sensitization.

Interpretation

The following findings strongly suggest that a newborn infant with hemolytic disease is the offspring of an Rh-sensitized mother:

(*1*) Rh-positive infant and Rh-negative mother.

(*2*) Rh antibodies in the maternal blood.

(*3*) Incompatibility of the mother's serum with the infant's cells which is not due to incompatible blood groups.

Findings which indicate that Rh antibodies are not the cause of hemolytic disease are:

(*1*) Rh-negative infant.

(*2*) Compatibility of the mother's serum with her infant's erythrocytes.

The following bloods are "not suitable" for transfusion in hemolytic disease of the newborn:

(*1*) Rh-negative maternal blood, unless plasma is removed by washing with saline.

(*2*) Rh-positive blood from any source, including paternal blood.

(*3*) Any Rh-negative blood shown to be incompatible by direct matching tests.

The following bloods are "suitable" for transfusion of infants with hemolytic disease of the newborn:

(*1*) Rh-negative blood of group O (in emergencies), possibly with the addition of factors A and B.

(*2*) Rh-negative blood of the infant's blood group.

(*3*) Rh-negative blood compatible with the mother's serum.

The "ideal" blood for transfusion of erythroblastotic infants is any blood designated as "suitable" when proved to be compatible by direct matching tests.

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CHAPTER XI

Renal Function Tests

Generally speaking, the function of the kidneys is to excrete waste products dissolved in water (1). Kidneys which function normally eliminate waste products in just the amount of water to maintain the body's water balance. If retention of wastes has been avoided but at the expense of body water, or if the attempt to maintain water balance has resulted in retention of waste, the mechanism may be considered as having failed (1). Over and beyond these two functions, the kidneys also maintain the reaction of the blood, by means of selective retention of base and by formation of ammonia.

The kidney's capacity to vary the composition of the urine in compliance with metabolic demands is explained by the concept of urine formation which is based on Cushney's theory (2). The concept is that water and crystalloids are removed from the blood in the glomeruli by simple filtration. Rehberg (3) summarizes the fate of the glomerular filtrate as follows: During its passage along the tubules, the filtrate loses 90 per cent of its water. A number of so-called "high threshold" substances, such as glucose, amino acids, chlorine, sodium, potassium, magnesium, and calcium, are largely reabsorbed along with the water. These substances are normally almost absent from the urine, but will appear in greater amounts when their concentration in the blood, and in the glomerular filtrate exceeds their normal renal threshold. Other substances, such as urea, uric acid, sulfates, and phosphates, are "low threshold" substances; they pass back by diffusion through the tubular wall into the circulation and are found in varying concentrations in the urine of normal subjects. "Nonthreshold" substances, such as creatinine, are held back in the intratubular concentrate and are excreted in the urine.

Apart from such substances as ammonia and hippuric acid, which are produced and excreted in the distal parts of the tubules, active tubular secretion, according to Best and Taylor (4), "is probably of relatively little importance under ordinary circumstances . . . but in chronic kidney disease it may be called upon to play a much more important role." This is particularly true of creatinine.

Smith's widely quoted experiments (5a) give an excellent idea of the magnitude of the work involved in normal renal function. In adults, approximately 125 cc. of fluid are filtered per minute, or 200 liters in 24 hours. Only 1 per cent of this amount is eliminated as urine, 99 per cent (198 liters) being returned to the blood.

Testing of renal function may be approached in a number of ways. The first and oldest is the chemical and microscopic analysis

TABLE 55

Kidney Function Tests

Test method	Individual functions studied	Structural units participating
Volhard's test.........	Concentrating and diluting power	Tubules
Fishberg test..........	Concentrating power	Tubules
Mosenthal's test*......	Concentrating power	Tubules
Pituitrin test*.........	Concentrating power	Tubules
Freshet test...........	Diluting power	Tubules
Phenolsulfonphthalein test*...............	Excretory power	Glomeruli and tubules
Urea clearance test*...	Glomerular filtration and tubular absorption	Glomeruli and tubules
Creatinine clearance test	Glomerular filtration and tubular secretion	Glomeruli and tubules
Inulin clearance test (mannitol clearance test)...............	Glomerular filtration (rate of formation of glomerular filtrate)	Glomeruli
Diodrast clearance test (*p*-aminohippurate clearance test)......	Effective renal blood flow (sum of excretion by glomerular filtration and by tubular secretion)	Glomeruli and tubules
Glucose clearance test..	Functional tubular mass (maximum ability of tubular cells to reabsorb glucose)	Tubules

* Described in the text.

of urine for products significant of kidney disease. The procedures for these tests are the same in children as in adults.

One may also have recourse to blood chemistry studies. In these, tests are made for abnormally increased retention of nitrogenous products, urea, phosphates, sulfates, and other substances. As an index of renal function, such tests are particularly important in moderate and severe forms of chronic nephritis. Slight insufficiencies, however, will escape detection, since retention occurs only after about two-thirds of the functional renal mass has become incapacitated.

Finally, a more direct means of testing the working capacity of the kidneys is to determine the efficiency of their partial functions: (*a*) ability to concentrate or dilute urine; (*b*) capacity to excrete chemical substances; (*c*) synthesizing power; (*d*) ability to maintain an effective blood flow; (*e*) ability to conserve base. A differential diagnosis of the morphologic pathology, however, cannot be made by means of function tests, even if they point to the anatomic site of the functional disturbance.

Only this last group of true function tests will be considered here. They are more precise and more sensitive than blood and urine tests, and some reveal functional impairment even during the earliest changes caused by anatomic renal disease. Table 55 lists the tests which can be regarded as representative of this group. Some of the procedures are old and in common use, others are new and just assuming clinical importance. Only the ones which seem definitely useful in pediatric practice will be described.

Renal function tests are valuable in two sets of circumstances. In acute and chronic nephritis, when the diagnosis of renal disease has already been established, the tests are invaluable in determining the functional damage to the kidneys. Repeated at proper intervals, such tests indicate the tendency of the disease and may serve as a check on the efficacy of therapy.

Function tests may also be of great service in establishing an early diagnosis of renal disease, for instance, in the course of extrarenal uropathies, in which the danger of renal involvement is a constant threat. When function tests are regularly employed in these conditions, renal impairment may be recognized sooner and more safely than when routine analysis of urine and blood only are used. The same holds true in other cases, such as acute and chronic nutritional disorders, with potential secondary involvement of the kidneys.

In choosing the most suitable test method, several factors should be kept in mind. Concentration and elimination tests are sufficiently sensitive to detect definite impairment of renal function, i.e., qualitative changes from normal to abnormal; but they are not so accurate as to reveal minor deviations from normal. These tests may give negative results when impairment of kidney function amounts to less than 30 per cent. Dilution tests yield even less reliable results. The urea clearance test is the most accurate approach to a precise quantitative measurement of renal efficiency. The study of inulin and diodrast clearances in children, according to Ranney and McCune (5b), "remains a research instrument and is not a clinical tool." These tests will therefore be discussed only very briefly.

In the final analysis, the question as to which test is indicated must be answered by an evaluation of the whole clinical picture. Concentration and elimination tests, for instance, are more likely to provide satisfactory information in uncomplicated cases of acute nephritis than in the study of chronic conditions, especially in their late stages. The age of the child is another important factor in selecting the appropriate test. The urea clearance test is the method of choice for infants and young children; it is the only method by which normal data for this age group have been secured.

It is unnecessary to subject every child with albuminuria and urinary casts to function tests. Only when clinical considerations call for additional evidence and measurement of renal dysfunction should such tests be used. Function tests should never be considered a substitute for careful clinical observation.

TWO-HOUR RENAL TEST (MOSENTHAL'S TEST)

This is one of the concentration tests. According to Mosenthal (6), it measures the tubules' capacity to vary urinary concentration of solids and to alter urinary volume. The specific gravity of the urine is determined in several 2 hour samples of urine on the day of examination, the variation in specific gravity serving as an index of the kidneys' concentrating power. A fixed specific gravity in all samples indicates loss of concentrating power and severe impairment of renal function.

PEDIATRIC CONSIDERATIONS

For children, the test's chief advantage over other concentration tests is the fact that water is not restricted and no rigid test diet is required. Other concentration tests require a period of water deprivation lasting 12 hours to 3 days. In young children this involves the risk of dehydration and acidosis. The Mosenthal test, however, may be done safely even in dehydrated children. The main obstacle to accurate testing of young children is the need for repeated collection of 2 hour urine samples. In this age group, it is difficult, even with the help of a trained nursing staff, to obtain emptying of the bladder at stated intervals without resorting to catheterization. This is probably why the test has been used almost exclusively in children old enough to exercise voluntary control. Normal values are available only for the 6 to 12 year old age group.

PROCEDURE

The method described is Hill's modification (7) of the Mosenthal test (6). On the test day, the child's breakfast should consist of 2 tablespoonfuls of cereal, 1 slice of bread, 2 tablespoonfuls of apple sauce, ½ square of butter, 6 ounces of milk, 4 ounces of water, 1 extra gram of salt, and 2 grains of caffeine sodium benzoate. Dinner is made up of 2 tablespoonfuls of chopped meat, 1 egg, 1 potato, 1½ squares of butter, 6 ounces of milk, 4 ounces of water, 1 extra gram of salt, and 2 grains of caffeine sodium benzoate.

No fluids other than the amount specified with each meal are given, except during the night, when some water may be given to relieve discomfort.

Starting at 6 A.M., urine specimens are collected at 2 hour intervals, the last one covering the period from 5 to 6 P.M. The urine is also collected from 6 P.M. to 6 A.M., and its quantity is measured. The samples collected during the day are tested as soon as they are obtained or at the completion of the test period. If the samples are not tested immediately, they should be stored in the refrigerator. Accurate collecting of specimens is essential; the bladder should be emptied each time, and the intervals should be exactly 2 hours each.

The specific gravity of each urine sample is determined with the urinometer. Saxe's urinopyknometer, which uses only 4 cc. of urine, is most convenient when only small quantities of urine are available. Since all urinometers are calibrated for use at specific temperatures, correction must be made for the room temperature. For every 3 degrees Celsius of the temperature above or below the calibration, 0.001 has to be added to or subtracted from the urinometer reading. An additional 0.003 must be subtracted for each per cent of protein, if a considerable amount of protein is present in the sample.

INTERPRETATION

The following are considered normal results in children between the ages of 6 and 11 years: (*1*) Considerable variation in the specific gravity of the various specimens. (*2*) A difference of at least 8 points between the highest and the lowest specific gravities. (*3*) A specific gravity over 1.020 in the night specimen.

Abnormal results for this age group are: (*1*) A fixed specific gravity in all samples. (*2*) A difference of less than 8 points between the highest and lowest specific gravities. (*3*) A specific gravity below 1.015 in the night specimen, or the presence of nocturnal polyuria; however, both of these signs are so variable in children that no great reliance should be placed on them. Repetition of the test during a prolonged illness may be of considerable prognostic value.

TABLE 56

Two-Hour Renal Test

Urine samples		Specific gravity of urine of	
		normal child, 6 years	child with acute nephritis, $3^2/_3$ years
6 A.M. to	8 A.M.	1.025	1.010
8	10	1.032	1.014
10	12	1.035	1.013
12 P.M.	2 P.M.	1.026	1.015
2	4	1.027	1.011
4	6	1.029	1.013
6	6 A.M.	1.029	1.013

From Hill (7).

Table 56 gives normal and abnormal results, as obtained in a healthy child and in a patient with acute nephritis, respectively.

PITUITRIN CONCENTRATION TEST

As originated by Leberman (8a), posterior pituitary extract is used to induce secretion of more concentrated urine in lieu of the water restriction required by the concentration tests of Volhard (8b) and Fishberg (8c). The kidneys' capacity to respond to the action of pituitrin by an increase in the tubular reabsorption of water is revealed by the changes in the specific gravity of the urine excreted after administration of the extract.

PEDIATRIC CONSIDERATIONS

The test compares favorably with other concentration tests and at the same time avoids fluid restriction, which is potentially dangerous in children. However, there are some risks in administering pituitrin to infants and children (page 394). Furthermore, the hourly collection of urine for 3 hours in young children presents the same technical difficulties as the Mosenthal test. Hence, no thorough enough studies with this test have been made in infants and young children, and the test therefore cannot be recommended for use in early childhood. It is satisfactory only for older children, in whom technic and interpretation are the same as in adults.

PROCEDURE

The method described is that of Schneeberg, Likoff, and Rubin (9a). Neither special diet nor restriction of fluid or food is required. On the test day the patient empties his bladder at a designated hour (specimen 1), after which 0.5 cc. (10 units) of surgical pituitrin is injected subcutaneously. Thereafter, urine is collected every hour for 3 hours (specimens 2, 3, 4). The specific gravity of all 4 specimens is measured with a clinical urinometer calibrated to a standard temperature. Corrections must be made for room temperature and protein content of the urine (page 364).

INTERPRETATION

If the specific gravity of specimen 1 ranges above 1.020, renal function is presumed satisfactory, and the rise in the specific gravity of specimens 2–4, as a result of the pituitrin, is only slight (9b).

If the specific gravity of specimen 1 is below 1.019, the normal response to pituitrin is an increase in the specific gravity to 1.020 or more in at least one of the other specimens. Failure to show such a concentration is interpreted as an abnormal response.

PHENOLSULFONEPHTHALEIN (PHTHALEIN) TEST

The test of Rowntree and Geraghty (10) measures the renal capacity to eliminate the dye phenolsulfonephthalein, which is not ordinarily contained in food. A standard dose of the dye is administered parenterally, and the amount of the dye excreted in the urine during the following 2 hours is determined. The result is expressed as a percentage of the injected dose excreted in the 2 hour period. The higher the percentage, the more efficient is the excretory function of the kidney.

PEDIATRIC CONSIDERATIONS

To obtain reliable test results in children, all the urine produced during the 2 hour period must be collected, and the bladder emptied at the end of the second hour. It should also be borne in mind that the response of the normal child to the test is not the same as that of the adult; a dye return considered normal in most adults should be regarded as abnormal in children. The diagnostic significance of the test in children is somewhat limited: a normal test reaction does not rule out functional impairment of the kidneys, particularly in children with chronic nephritis. Normal excretion of the dye has been found when other functional tests have revealed severe renal damage. The test is therefore useful in children only when used together with concentration tests.

PROCEDURE

Reagent. Test dye solution. To 600 mg. phenolsulfonephthalein in a 100 cc. volumetric flask add 0.84 cc. of 2 *N* sodium hydroxide, dissolve the dye with the aid of 0.75 per cent sodium chloride solution, add a further 0.15 cc. of 2 *N* sodium hydroxide, and dilute to volume with the 0.75 per cent sodium chloride solution. Ampules (1.1 cc) of sterile solution are commercially available.

According to the directions given by Hill (7), neither fasting nor dietary restriction is required before or during the test. Sometime between breakfast and lunch, 6 mg. of phenolsulfonephthalein, contained as the sodium salt in 1 cc. of the above test solution, are injected intramuscularly. All urine passed during the next 2 hours is collected, including whatever may still be in the bladder at the end of the second hour.

The dye content of the urine specimen thus obtained, representing the entire 2-hour output, is determined as follows: The urine specimen is transferred into a 1-liter volumetric flask, and 10 per cent sodium hydroxide is added slowly, with stirring, until the red color of the alkaline phenolsulfonephthalein has reached its full intensity. The specimen is then diluted to 1 liter with distilled water. The intensity of the red color is compared in a colorimeter with that of a standard solution of the dye, which contains (*1*) 0.5 cc. of the 0.6 per cent phenolsulfonephthalein solution (3 mg., or half the amount used for injection); (*2*) 5 cc. of 10 per cent sodium hydroxide; (*3*) water or saline to make 1 liter. The strength of this standard is designated as 50 per cent. The percentage of the injected dye eliminated in the urine is calculated by the formula:

$$\frac{\text{Reading of urine (unknown)} \times \text{strength of standard (in \%)}}{\text{reading of standard}}$$

If the color of the urine is too intense to be compared with the 50 per cent standard, a 100 per cent standard is made up by diluting 6 mg. (1 cc. of the 0.6 per cent dye solution) to 1 liter.

INTERPRETATION

The average normal return of the dye in children between the ages of 2 and 8 years is about 75 per cent of the amount injected. This is definitely higher than in adults, who show an average normal elimination of about 65 per cent.

A value below 60 per cent in a child under 8 years of age may be considered abnormal.

In children over 8 years, the borderline between normal and abnormal responses is the same as in adults, namely, around 50 per cent.

Normal test results do not prove that renal function is intact. Hill (7) states: "In dealing with children, normal or high phthalein excretion helps us very little, as the test may be high in many cases in which there is unquestionably severe kidney damage."

A low excretion of the injected dye is more revealing. It is invariably a sign of subnormal kidney function. Such a result is found in children in the acute stages of nephritis, the average elimination of the dye being 59 per cent. In the chronic phase of nephritis, which is almost always due to infection, the excretion is usually not delayed. An excretion of 40 per cent or less, such as typically occurs in adults in chronic vascular kidney disease, is extremely rare in children.

In orthostatic albuminuria the response to the test is without exception abnormal. When accentuated lordosis is maintained in the lying or sitting position during the test, retarded and decreased dye excretion is shown by children with orthostatic albuminuria, but not by normal individuals (11).

Fractional Phthalein Test. Urine is collected every 15 minutes for 1 hour after intravenous or intramuscular administration of the standard test dose of the dye, and the dye concentration is measured in each of the 4 samples. The same technic is used as in the test above.

Normally, with intravenous injection of the dye, the peak of excretion is found in the first specimen. With intramuscular administration, the dye is excreted more slowly, the peak being reached in the second and third specimens of urine (12).

In children between the ages of 4 and 13 years, the normal excretion values are similar to those of adults. The test appears to be more useful for urologic examination, i.e., to ascertain the separate functions of the ureter and kidneys, than in general pediatric practice.

UREA CLEARANCE TEST

Attempts to develop a better understanding and more accurate measurement of urinary secretion have been furthered by the use of mathematic formulas, and have led to Van Slyke's conception of urea clearance (13). This in turn led to Smith's elaboration of inulin and diodrast clearance tests (page 377).

The formulas used in the urea clearance test are valid only when the relation of the urea clearance rate to the urinary volume and to the blood urea level is governed by a constant. The existence of such a constant has been verified by Van Slyke and his associates: The rate of urea excretion is directly proportional to blood urea concentration when the urine volume is at least 2 cc. per minute in normal adults. This constant is termed the "augmentation limit." At or above this limit "the minute output of urea represents the maximal quantity of blood which is cleared of urea per minute." This is termed the "maximum clearance" (C_m). When the volume of urine is below the augmentation limit, i.e., less than 2 cc., the blood clearance of urea varies directly as the square root of the urine volume and is called "standard clearance" (C_s). The formulas for both clearances are given below.

Average normal maximal and standard clearances for adults with a mean body surface area of 1.73 square meters have been established, and it is now customary to express test results as a percentage of these established normals.

The test is an extremely sensitive one; no other test is as reliable in detecting impaired renal function. A 40 to 50 per cent reduction in urea clearance may occur before the blood urea rises above normal values.

Formulas. (*1*) With a urine volume of 2 cc. or more per minute:

$$C_m = (U \times V)/B$$

(*2*) With a urine volume of less than 2 cc. per minute:

$$C_s = (U \times \sqrt{V})/B$$

where U is milligrams urea nitrogen per 100 cc. urine; V is volume of urine, in cubic centimeters per minute; and B is milligrams urea nitrogen per 100 cc. blood.

PEDIATRIC CONSIDERATIONS

As shown by Van Slyke and his co-workers (13), the laws governing the rate of urea clearance from the blood in adults apply to children as well, and normal clearances in children are of the same order as in adults. This has been corroborated by other investigators. Consequently, urea clearance in children may be measured and calculated by the same method as in adults, provided the child is at least 2 years old, and that correction is made for the child's surface area so that the results always express urea clearance referred to the standard surface area.

A somewhat different method of computing urea clearance and of evaluating the figures is necessary in children below the age of 2 years. As has only recently been discovered, urinary secretion during the first year of life differs physiologically from that of older children. The relation between urinary flow and urea excretion is apparently not as constant as it is in adults, which renders the basis for differentiation between standard and maximum clearances rather doubtful in young children. For this reason, urea clearances in infants are, as a rule, considered as maximal clearances, and the use of the standard clearance and its square root radical is omitted.

Furthermore, according to McCance and Young (14), in infants "urea clearance is very low by adult standards whether compared on the basis of surface area, kidney mass, or body weight." The younger the infant, the lower his normal urea clearances. Obviously, figures of average normal clearances in children over 2 years and in adults are not valid for the younger age group. Normal values for infants are now available for comparison.

The use of the urea clearance test in children is not without some technical problems. In small children it is difficult to collect the urine for 2 successive 1-hour periods, and assure complete emptying of the bladder at the end of the second hour; the bladder must be catheterized and washed out, if the results are to be accurate. The intake of the relatively large amounts of fluid which the test requires is also difficult to carry out in infants. The modification of Landis, Elsom, Bott, and Shiels (15) somewhat reduces these difficulties. It is a 24 hour clearance test, with long periods of urine collection and a minimum of interference with the infant's regular feedings.

Urine and blood analyses provide the actual data for the calculation of urea clearance. The urine is analyzed for urea nitrogen and ammonia nitrogen; their sum represents the true urea nitrogen, on the assumption that all urinary ammonia has passed the glomeruli as urea. Instead of estimating the two nitrogens separately, "urinary nitrogen," which represents approximately 95 per cent of the sum of urea and ammonia nitrogens, can be conveniently determined by the hypobromite method (16).

Blood urea is estimated in one blood sample only. Comparative studies have shown that the result when the fasting urea value alone is obtained is as accurate as the average figure for urea obtained from both morning and night blood samples (15). With Farr's method (17), only 0.25 cc. of blood is needed.

The rapid hypobromite method, using the manometric apparatus, described below, is a convenient method of estimating urea in blood and urine. Textbooks of quantitative chemistry, for example, Peters and Van Slyke (13), may be consulted for the technic and application of other analytic procedures.

PROCEDURES

The 24 hour clearance test (15) is recommended for all children up to the age of 2 years, as well as for older children if it facilitates performance of the urea clearance test.

The infant or child is fed his usual diet, but the total quantity of food should be given in 6 to 8 aliquots at 3 to 4 hour intervals during the 24 hours. No additional fluids are required. Height and weight are determined at the beginning of the test. Urine is collected as accurately as possible for 24 hours, usually from 8 A.M. to 8 A.M. Small losses must be recorded, although they fail to introduce any considerable error in the final result. Metabolic frames, or any other device which is available for urine collection, may be used for small children. Catheterization is contraindicated. The urine is stored in the refrigerator, with toluol as a preservative, until the collection is complete. At the end of the 24 hours the total amount of urine is well mixed, its volume measured, and its urea content estimated in an aliquot portion.

A sample of capillary blood is collected before the first feeding in the morning. The 0.25 cc. needed for the determination of urea can be taken from finger or heel with a micropipet, like those used for blood sugar determination.

Estimation of Urea in Urine. The method described is that of Van Slyke (16).

Reagent. Hypobromite reagent, which consists of: (*1*) Bromine solution. Dissolve 60 mg. of potassium bromide in 100 cc. of water, and then dissolve 2.5 cc. of bromine in the potassium bromide solution. If the solution is kept in a dropping bottle, the amount needed for each analysis can be measured out from the pipet stopper. (*2*) Sodium hydroxide solution. Dissolve 40 Gm. of sodium hydroxide in water and bring to 100 cc.

Apparatus. Van Slyke-Neill gasometric apparatus.

Technic. The urine is diluted tenfold, or twentyfold if a very high concentration of urea is anticipated. Then 2 cc. of the diluted urine are either layered under the water or pipetted through it into the chamber of the Van Slyke-Neill apparatus. The pipet is withdrawn and the water is run into the chamber. The hypobromite reagent is then added by transferring 1.25 cc. of the 40 per cent sodium hydroxide into the cup at the top of the chamber, adding 0.75 cc. of the bromine solution, and mixing the two; 1.5 cc. of the mixture are then run into the chamber and the cock is sealed with a drop of mercury. The mercury in the chamber is at once lowered to the 50 cc. mark, and the chamber is shaken for 1.5 minutes if the temperature is 25 C., 2 minutes if the temperature is 20 C., and 3 minutes if the temperature is 15 C. The volume of gas is reduced to 2 cc., and the pressure (p_1) is read on the manometer.

A blank analysis, with 2 cc. of water replacing the urine, is run, and the manometer reading is taken (p_0).

If the temperature in the water jacket of the apparatus rises between the time of the urinalysis and that of the blank, 1.3 mm. are added to the reading of the blank (p_0) for each degree of temperature increase. A similar correction is made if the temperature falls, by subtracting from the reading of the blank.

TABLE 57

Factors by Which Millimeters P_{N_2} Are Multiplied to Give Grams Urea Nitrogen per 100 cc. of Urine

Temp., C.	Urine sample analyzed		Temp., C.	Urine sample analyzed	
	0.1 cc. a = 2.0 cc.*	0.2 cc. a = 2.0 cc.*		0.1 cc. a = 2.0 cc.*	0.2 cc. a = 2.0 cc.*
15	0.00312	0.001561	25	01	06
16	11	55	26	00	00
17	10	49	27	0.00299	0.001495
18	09	44	28	98	90
19	08	38	29	97	85
20	07	33	30	96	80
21	05	27	31	95	74
22	04	22	32	94	69
23	03	16	33	93	64
24	02	11	34	92	59

From Van Slyke (16).
* Volume at which gas pressure in the Van Slyke-Neill apparatus was read.

The chamber of the apparatus need not be washed between successive analyses of a series.

Calculation. The pressure of liberated nitrogen is:

$$P_{N_2} = p_1 - p_0$$

The urea nitrogen content of the urine is calculated as:

$$\text{Grams urea N per 100 cc. urine} = P_{N_2} \times \text{factor}$$

The values of the factor are given in Table 57.

Estimation of Urea in Blood. The method described is that of Farr (17) and of Van Slyke and Kugel (18).

Reagent. Hypobromite reagent (page 371).

Apparatus. Van Slyke-Neill gasometric apparatus.

With a micropipet 0.25 cc. of blood is taken from finger tip or heel, and transferred and laked in 12 cc. of distilled water which has been measured with a volumetric pipet into a conical centrifuge tube. The laked blood is stirred, and 0.25 cc. of 17 per cent ferrisulfate ($FeSO_4.H_2O$) is added and thoroughly mixed with a stirring rod. Then 0.2 to 0.4 Gm. of solid barium carbonate is added; this is done most conveniently by using a small ladle which has been previously measured to contain the correct weight of barium carbonate. An excess does not affect the analysis. The mixture is stirred until a precipitate begins to flocculate; settling is rapid and the supernatant liquid should be clear. If there is a yellowish tinge, additional barium carbonate should be added, and the contents mixed thoroughly. The tube is then centrifuged for fifteen minutes at 2,500 r.p.m., and the contents are filtered through a 5 × 7 cm. filter paper into a 25 cc. Erlenmeyer flask. Usually 10.5 to 11 cc. of filtrate are obtained, which is enough for a duplicate analysis. With an Ostwald bulb pipet, 5 cc. of the filtrate, representing 0.1 cc. of the original blood sample, are transferred to the chamber of the Van Slyke-Neill apparatus. The chamber is sealed, evacuated, and shaken for 2 minutes, to free the solution of dissolved gases. After these are expelled, 1.5 cc. of the hypobromite reagent are run into the chamber, as has been described above for the analysis of urine. The chamber is again sealed, evacuated, and shaken for about 1½ minutes at ordinary room temperature. The pressure of the gas evolved is measured, with the gas occupying a volume of 0.5 cc. with ordinary urea content. If the urea content is so high that the pressure at 0.5 cc. exceeds 400 mm., the gas is brought to a volume of 2 cc. The reading on the manometer is designated as p_1.

The gases are then completely expelled, the cock is sealed with mercury, the solution meniscus is lowered to the mark at which it stood when the p_1 reading was taken, and the p_0 reading is made.

A blank analysis is done, with 5 cc. of 0.62 per cent sodium chloride solution replacing the blood filtrate. The difference between the first reading, b_1, and the second reading, b_2, as determined in this blank analysis, is the correction (c) of the analysis. Its absolute value is 6 to 8 mm. when the pressure is measured with the gas at 0.5 cc. volume, and 1.5 to 2 mm. at 2 cc. volume.

TABLE 58
Factors by Which Millimeters P_{N_2} Are Multiplied to Give Milligrams Urea Nitrogen per 100 cc. of Blood

Temperature, C.	Blood sample analyzed			
	0.5 cc.		0.1 cc.	
	a = 0.5 cc.*	a = 2.0 cc.*	a = 0.5 cc.*	a = 2.0 cc.*
15	0.1592	0.636	0.7960	3.180
16	86	34	930	70
17	80	32	900	60
18	75	30	875	50
19	69	27	845	35
20	63	25	815	25
21	58	23	790	15
22	52	21	760	05
23	46	18	730	3.090
24	41	16	705	80
25	36	14	680	70
26	30	12	750	60
27	25	10	625	50
28	20	08	600	40
29	15	06	575	30
30	09	04	545	20
31	04	02	520	10
32	0.1498	00	490	00
33	93	0.598	465	2.990
34	89	96	445	80

From Van Slyke and Kugel (18).
* Volume at which gas pressure in the Van Slyke-Neill apparatus was read.

Calculation. The pressure P_{N_2} obtained in the blood analysis is calculated by the formula:

$$P_{N_2} = p_1 - p_0 - c$$

The urea nitrogen content of the blood is calculated as:

$$\text{Mg. urea N per 100 cc. blood} = (P_{N_2} \times \text{factor}) - 1$$

The empiric correction of subtracting 1 is not made if the uncorrected value is less than 10 mg. per hundred cubic centimeters.

The values of the factor are given in Table 58.

Calculation of the Urea Clearance. The following data must be determined by analysis or computation:

(*1*) Milligrams urea N per 100 cc. blood (*B*).

(*2*) Milligrams urea N per 100 cc. urine (*U*).

(*3*) Cubic centimeters urine excreted per minute (*V*).

(*4*) Square meters of surface area (*S.A.*); this is calculated either from height and weight according to the DuBois and DuBois formula (page 83), or, for infants, by using the formula (19):

$$5.188 \times \text{weight}^{0.75}$$

From the figures thus obtained, the urea clearance is calculated as follows.

(*A*) *In children 2 years of age* and over the formulas for maximal clearance and standard clearance in adults (page 369) are used, corrected for body size. This correction is made by multiplying *V* by the quotient 1.73/*S.A.*, since the formulas are valid only for an average surface area of 1.73 square meters.

The corrected formula for maximal clearance, with $V(1.73/S.A.)$ equal to 2 or more, reads:

$$C_m = \frac{U}{B} \times V \times \frac{1.73}{S.A.} = \text{cc. blood cleared of urea per minute.}$$

The corrected formula for standard clearance, with $V(1.73/S.A.)$ equal to less than 2, reads

$$C_s = \frac{U}{B} \times \sqrt{V \times \frac{1.73}{S.A.}} = \text{cc. blood cleared of urea per minute.}$$

Results thus obtained may be compared with the average normal "standard" and "maximal" clearances, respectively (Columns 1 and 4, Table 59).

(*B*) *In children under 2 years of age* it is most convenient to express results of the test in terms of minute volume (cc./min.) per square meter, instead of per 1.73 square meter, so that 1/*S.A.* replaces 1.73/*S.A.* in the above formula, and the formula reads:

$$\frac{U. \times V.}{B. \times S.A.} = \text{cc. blood cleared of urea per sq. meter per minute.}$$

Comparison with normal figures (Column 5, Table 59) for the corresponding age group will reveal whether the clearance is reduced or not.

TABLE 59

Normal Urea Clearance in Infants and Children

1	2	3	4	5	
Age, postnatal*	Uncorrected clearance (average), $\frac{U \times V}{B}$	Maximum clearance (average), $\frac{U \times V \times 1.73}{B \times S.A.}$	Standard clearance (average), $\frac{U}{B} \times \sqrt{V \times \frac{1.73}{S.A.}}$	Clearance, $\frac{U \times V}{B \times S.A.}$	
				Range	Average
	cc./min.	cc./1.73 sq. m./min.		cc./sq. m./min.	
Newborn, premature, 2–18 days (20)	1.7	—	—	2.2–9.5	5.8
Newborn, full term, 7–10 days (14)	—	—	—	5–19	7.0
Infants, premature, 8–65 days (21)	2.8	—	—	8.5–24.1	15.3
Infants, full term, 5–8 weeks (14)	—	—	—	10–27	18.5
7–73 days (21)	5.6	—	—	13.8–31.3	21.0
4–7 months (14)	—	—	—	17–32	24.5
2–12 months (22)	—	73	50.5	23–55	38.0
Children, 2 years and over, and adults (23)	—	75	54	30	40

* Numbers in parentheses are reference numbers.

INTERPRETATION

Average normal figures are given in Table 59. Urea clearance in the infant is physiologically defective. It increases steadily from birth to the second year, when normal adult values are reached. This clearing defect is even greater in premature infants. Gordon and Levine (24) state: "Such impairment of renal function must handicap the young infant in his selective excretion of water and solids under stress."

While abnormally low clearances are significant of damaged kidney function, they do not indicate whether it is glomerular or tubular dysfunction which is responsible for the impaired excretion of urea.

Urea clearance in children is definitely influenced by other than renal factors as well. To name the most important ones, dehydration and protein intake both affect the clearance. Dehydrated infants show an abnormally low urea clearance during the acute stage of severe nutritional disorders; and urea clearance increases with high protein intake, declines with low protein intake.

INULIN AND DIODRAST CLEARANCES

Inulin is a polysaccharide (25); it is built up entirely from fructose, which occurs as a reserve substance in many plants. Inulin dissolves fairly easily in water, producing a colloidal solution.

According to Smith (26,27), the originator of the tests in question, inulin is not metabolized in the body. It is excreted solely by glomerular activity, the tubules neither reabsorbing nor excreting the substance. Under all experimental conditions, inulin clearance has been found to be equal to the rate of glomerular filtration. In contrast to urea clearance, which is the result of the combined activity of the glomeruli and tubules, inulin clearance is the result of glomerular activity alone.

Substances with a higher excretory rate than inulin are not cleared from the blood by glomerular filtration alone, but by tubular excretion as well. Lower clearances than that of inulin can only be explained by tubular reabsorption. The following illustrates how the inulin clearance test may serve to measure specific glomerular function:

A patient with nephritis, let us say, shows a maximum clearance of urea which is 25 per cent below the normal value. If the inulin clearance shows a corresponding drop, it may be assumed that the functional impairment of the kidneys is due predominantly to glomerular damage. If, however, the patient's ability to clear inulin from the plasma is essentially intact, the impaired urea clearance is evidently the result of tubular damage.

The plasma clearance of one of the hexitols, for example, mannitol, may be used in similar fashion to measure glomerular filtration (28).

The diodrast clearance test has been devised by Smith (26,27) as a means of demonstrating and defining tubular dysfunction. Diodrast is an iodine compound, containing approximately 50 per cent iodine. The commercially available preparation is a solution of diethanolamine and diiodopyridineacetic acid. Diodrast does not enter the body tissues or the blood cells; when present in low concentrations, it is completely removed from the plasma by a single passage of the blood through the kidneys. Under normal conditions, only 16 per cent of the diodrast is cleared by glomerular filtration, the remainder being excreted by the tubules. Diodrast clearance measures the "effective renal blood flow," i.e., the amount of blood which has passed through active renal tissue per unit of time. If both rate of glomerular filtration (measured by inulin clearance) and the effective blood flow (measured by diodrast clearance) are known, the factor responsible for altered renal function can be identified. For instance, the high urea clearance of nephrotic children can be defined as the result of an increase in both glomerular filtration and renal blood flow, since inulin as well as diodrast clearances have been found to be equally high in such children (29).

Recently, *p*-aminohippuric acid has been introduced as a substitute for diodrast (30).

The "functioning tubular mass" can be investigated separately as to its excretory and absorptive capacities. As a measure of the maximum secretory ability of the tubules, diodrast or *p*-aminohippuric acid clearance is determined. Correspondingly, the clearance of glucose by tubular absorption is used as an index for the maximum reabsorptive ability of the tubular mass (5a).

For further details and technical procedures, the reader is referred to the original publications (26,27).

ADDIS SEDIMENT COUNT

A patient subjected to a period of fluid restriction will excrete an acid and concentrated urine, and the sediment of such urine can be examined quantitatively for formed elements. Healthy individuals respond to dehydration with the excretion of relatively few casts and blood cells, whereas nephritic patients show a high increase in all formed elements. Addis (31) has established the values for normal healthy adults, and has correlated abnormally high figures with pathologic renal conditions.

As the appearance of morphologic elements in the urine represents an induced reaction, the examination, according to Addis, may be properly considered as a function test method.

PEDIATRIC CONSIDERATIONS

Rigid restriction of water intake, as directed in the original test method, has proved undesirable and unnecessary in children. The required urinary acidity of *p*H 5 to 6 and a specific gravity well above 1.020 can be attained by a shorter and less stringent period of water deprivation than is needed for adults. Normal values for children between the ages of 4 and 12 have been established with such a modified test regimen, and are available for comparison. Typical values for the acute and degenerative stages of nephritis in children have also been found. But it is still not definitely established whether abnormal Addis counts in children are evidence of anatomic pathology only, or of functional impairment as well.

PROCEDURE

The method described is that of Addis (31) as modified by Weiner and Schwarz (32). The child is given his usual dinner, including fluids up to a quantity of 200 cc., at 4 P.M. For the following 15 hours no fluids are allowed. From 7 P.M. to 7 A.M. urine is collected in a rubber-stoppered bottle, and the total amount is measured. The contents of the bottle are thoroughly mixed by repeated inversion, and a 10 cc. sample is transferred to a special centrifuge tube graduated in 0.1 cc. up to 10 cc. The tube is centrifuged for 5 minutes at 1,800 r.p.m., the supernatant urine is partially decanted, and then pipetted down to a known volume, which varies with the amount of sediment, as judged by direct observation. Usually, the urine is drawn off to exactly 0.5 cc. The sediment is thoroughly mixed in this remaining fluid by means of a capillary pipet and drops are transferred to a counting chamber (hemocytometer). The casts are counted under the low power of the microscope in the entire ruled area of 18 sq. mm. Red and white cells are counted in a unit of 8 sq. mm., using the high dry objective.

To make differentiation of red and white cells easier, Rew and Butler (33) have recommended the staining of the sediment with cresyl blue. For this two solutions are needed: (*1*) 0.964 Gm. sodium citrate ($Na_3C_6H_5O_4.H_20$), 0.589 Gm. of sodium chloride, and water up to 100 cc.; (*2*) a 1 per cent solution of cresyl blue in physiologic saline. To stain the sediment, 4.5 cc. of the sodium citrate–sodium chloride reagent and 4 drops of the freshly filtered dye solution are added to the sediment suspension in the centrifuge tube, after the centrifuged urine has been pipetted down to a volume of 0.5 cc. The mixture is stirred thoroughly with a capillary pipet, centrifuged, decanted, and drawn off to 0.5 cc., as before the dye was added. The microscopic examination then follows.

The number of elements is calculated from the formula:

$$\frac{\text{Number counted} \times \text{cc. of 12 hr. urine} \times 1{,}000}{\text{Cubic millimeters inspected} \times \text{concentration of sediment}}$$

Example. 15 red cells were counted in 8 sq. mm., or 0.8 c. mm.; the volume of 12 hour urine was 140 cc.; 10 cc. of urine were transferred into the centrifuge tube, sediment was concentrated to 0.5 cc., and its concentration amounted to 10/0.5, or 20. The number of red cells contained in the 12 hour specimen amounted to:

$$\frac{15 \times 140 \times 1{,}000}{0.8 \times 20} = 131{,}250$$

INTERPRETATION

Standard values of formed elements excreted during a 12 hour period by normal adults and children 4 to 12 years old are given in Table 60. The table also gives average values found in children with nephritis.

Sediment counts above the normal, but below the nephritic levels, are commonly to be found during the late stages of acute nephritis, indicating a latent but still active lesion. In the absence of clinical findings and albuminuria, the clinical significance of these slightly or moderately abnormal counts should not be overestimated. Many nephritics show this abnormal response to the Addis test long after complete clinical recovery. Such children should not be subjected to any restrictions, unless other tests prove that the functional capacity of the kidneys is impaired. When facilities for ac-

TABLE 60
Addis Count under Normal Conditions and in Renal Disorders*

	Normal young adults (31)		Normal children (34)		Nephritic children (35)	
Elements	Average	Upper normal	Average	Upper normal	Acute stage, average	Degenerate stage, average
Casts	1,040	5,000	1,085	10,000	544,000	1,880,000
Red blood cells	65,750	500,000	15,181	600,000	103,000,000	—
White and epithelial cells	322,500	1,000,000	320,000	1,000,000	182,000,000	9,900,000
Protein (albumin), mg	30	60	18.5	35	96	3,180

* Numbers in parentheses are reference numbers.

curate testing of kidney function are not available, the Addis test offers a relatively simple means for a rough check as to the course of late nephritic changes, and a recrudescence may be detected earlier than by clinical observation. But a persistently slight increase in formed elements, even during many months, should never form the sole basis for therapeutic measures and an unfavorable prognosis (36).

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CHAPTER XII

Nervous System

Only a limited number of test procedures, most of them concerned with the motor functions of the nervous system, are described in this chapter. The tests provide means for a differential diagnosis of neuromuscular disease. Electroencephalography is also considered, but ventriculography, pneumonencephalography, and tests showing changes in the spinal fluid have not been included in this discussion.

ELECTRIC TESTS OF NEUROMUSCULAR EXCITABILITY

Peripheral nerve-muscle, forming a functional unit, responds in typical fashion to stimulation both by faradic and by galvanic current. The character of the reaction and the degree of excitability serve as the criteria of normal or abnormal function.

With the monopolar method, the stimulating electrode is placed upon the skin overlying the motor point of the muscle (direct muscular test), or overlying the nerve trunk which enters the muscle more distally (nerve test or indirect muscular test).

In galvanic testing, the stimulating electrode can be used alternately as cathode or anode. Contractions are elicited only when the circuit is made or broken. Depending on whether the stimulating electrode acts as cathode or anode, four reactions are noted: (*1*) cathodal closing contraction (C.C.C.); (*2*) anodal closing contraction (A.C.C.); (*3*) anodal opening contraction (A.O.C.); (*4*) cathodal opening contraction (C.O.C.).

A quantitative and more exact method of electric testing is the examination of the chronaxia of a muscle or nerve (1), but since special knowledge and training are required for measuring chronaxia, this test method will not be discussed here. For the same reason, discussion of electromyography, a method for recording muscular function (2), is omitted.

PEDIATRIC CONSIDERATIONS

More than 20 years ago, when children with active or latent tetany formed a large group in pediatric clinics, the diagnosis of tetany was considered incomplete without confirmation by electric tests (Erb's sign). Tetany is today a rare disease in children, and with its growing rarity has vanished, as Powers (3a) puts it, the fine art of doing electrical reactions. In its stead, measurement of the serum calcium level is now the standard means for differentiating between tetany and disorders which resemble it. Nevertheless, electric hyperexcitability still remains the outstanding functional symptom of tetany; in the rare normocalcemic forms of tetany, it is still the chief diagnostic criterion (3b).

Electric testing is also used in children to identify other neuromuscular disease, such as dystonia, dystrophy, and paralysis.

PROCEDURE OF GALVANIC AND FARADIC STIMULATION

Any one of the commercially available apparatus may be used. The poles of the apparatus are connected to the two electrodes through which the current enters and leaves the patient's body. The small stimulating electrode is about 3 sq. cm. in size; to it is attached the device for opening and closing the circuit. The large, rectangular indifferent electrode is 15 to 20 sq. cm. in size.

In testing galvanic excitability, the stimulating electrode is first made to function as the cathode. It is placed upon the skin overlying the median nerve, within the distal part of the sulcus bicipitalis medialis. A positive response to stimulation of the median nerve consists of a flexing movement of the whole hand, or in short twitches of the flexor muscles of one or more fingers. A current of 4 milliamperes (ma.) is employed and the response to the closing of the current is observed. By gradually decreasing or increasing the strength of the current, the threshold of excitability (C.C.C.) is determined. The response to the opening of the circuit (C.O.C.) is tested in a similar manner. With the current reversed and the stimulating electrode now representing the anode, the examination is repeated both as to closing and opening reactions (A.C.C. and A.O.C.).

Finally, the muscular reaction to faradic stimulation is observed. Any nerve trunk and any muscle lying close enough to the surface may be tested in this way. The motor points of the most important muscles and nerves may be found in illustrations in neurologic textbooks, such as the one by Herz and Putnam (4a).

The physician needs the assistance of three persons when testing a young child or infant: one to hold the child in one position, another to keep the indifferent electrode in place on the patient's chest, and the third to serve as switchboard operator to regulate the current, read the electrometer, and record the results. The physician himself handles the stimulating electrode and observes the muscular response.

INTERPRETATION

The normal response to faradic stimulation is a prompt and strong tetanus-like contraction of the muscle which lasts as long as the current is flowing.

The four normal reactions to galvanic stimulation occur in the following order, as judged by the strength of current required for their elicitation:

$$\text{C.C.C.} < \text{A.C.C.} < \text{A.O.C.} < \text{C.O.C.} < \text{C. O. Tet.}$$

That is to say, the weakest current will elicit the cathodal closing current (C.C.C.). The appearance of the reactions in this order is one of the criteria of normality. Frequently, the cathodal opening contraction cannot be obtained with currents below 5 ma., and with increasing strength of current a tetanic contraction (C.O.Tet.) appears. The normal values of the other three reactions have a range between 2.5 and 5 ma.

Abnormal responses to electric stimulation may consist of: (*1*) increased excitability, or abnormally strong muscular response; (*2*) decreased excitability, or abnormally weak muscular response; (*3*) reversal of normal order of the 4 galvanic reactions; (*4*) abnormal character of contraction.

Increased response to the galvanic current occurs in all forms of *tetany*. The "cathodal hyperexcitability"—positive responses to cathodal stimulation with abnormally weak currents—is particularly significant. Elicitation of a cathodal opening contraction or of a tetanic contraction with currents below 4 ma. is considered pathognomonic of tetany. In addition to cathodal hyperexcitability, anodal reversal may be present; in which the anodal opening contraction is more easily elicited than the anodal closing contraction. Table 61 shows the range of normal neuromuscular reactions, as compared to responses obtained in tetany.

TABLE 61
Minimal Galvanic Currents Required to Elicit Neuromuscular Response in Normal Children and in Tetany

Condition	Response			
	C. C. C.	A. C. C.	A. O. C.	C. O. C.
	Milliamperes			
Normal......	0.9–2.5	1.8–4.0	3.0–6.0	>4.0
Tetany.......	0.1–2.0	0.3–3.0	0.7–4.0	0.7–4.0

Only in early childhood is galvanic hyperexcitability pathognomonic of tetany. Some children 2 years of age and over always show electric and mechanical hyperexcitability in the absence of other clinical or chemical signs of tetany. In such children the tetanoid pattern of neuromuscular reactions is not caused by an acute disturbance of the electrolyte metabolism, as it is in tetany, but is rather the expression of a vagotonia, or imbalance in the vegetative nervous system (5).

In *myotonia congenita* (Thomsen's disease), the most typical reactions are: increased excitability, particularly to faradic stimulation, and prolongation of the contraction after the electric circuit has been cut off.

In *myatonia*, the muscular reactions are not very significant, although a definite faradic hyperexcitability is rarely absent.

In *myasthenia gravis*, the majority of patients manifest *Jolly's reaction* (6). The reaction consists of an apparently normal response to direct faradic stimulation of the muscle for the first few seconds, followed by a gradual decrease in the contraction until all reactivity has ceased. Repeated stimulation elicits a strong response which disappears more rapidly than in the first test, and each repetition leads to progressive exhaustion and shortening of the contraction. In advanced stages of the disease, exhaustion is reached within 20 to 30 minutes. Galvanic irritability, however, persists to a considerable degree, although not in normal strength. The test is particularly valuable when muscles affected by the disease are such as can be conveniently tested.

The *reaction of degeneration* consists of a combination of quantitative and qualitative changes of the neuromuscular excitability (Table 62). In addition, there is reversal in the neuromuscular response to the galvanic current: anodal closing or anodal opening contractions are more easily elicited than cathodal closing contractions. Most characteristic is the sluggish, wavy, or vermicular contraction of muscles which still respond to the galvanic current.

TABLE 62

Reaction of Degeneration. Changes in Muscular Response Elicited through Indirect (Nerve) and Direct (Muscle) Electric Stimulation

Designation of reaction	Response to	
	Faradic stimulus	Galvanic stimulus
Normal reaction		
Nerve.......	Tetanus-like contraction	Brisk, single contraction
Muscle......	Tetanus-like contraction	Brisk, single contraction
Partial R. D.*		
Nerve.......	Diminished response	Diminished response
Muscle......	Diminished response	Sluggish contraction
Full R. D.*		
Nerve.......	No response	No response
Muscle......	No response	Slow, vermicular contraction
Absolute R. D.*		
Nerve.......	No response	No response
Muscle......	No response	No response

* R. D.: reaction of degeneration.

The reaction of degeneration is a criterion of impairment or loss of trophic connection between the neuron and the muscle. Trauma or other destructive pathology may be responsible for the severed link between nerve and muscle. Diseases of the neuromuscular system which lead to the reaction of degeneration and with which the pediatrician may have to deal, are poliomyelitis, spinal progressive muscular atrophy, and neural progressive muscular atrophy. The presence or absence of this reaction is an important factor in the differentiation between the spinal muscular atrophies and the so-called muscular dystrophies. The prognosis in poliomyelitis is greatly aided by electric tests of the paralyzed muscles; if a full or absolute reaction of degeneration persists longer than 6 months after onset of paralysis, there is little hope for functional improvement (7).

Charting of Muscular Paralysis. Attempts have been made to set up standards for evaluating residual damage to muscles in poliomyelitis. While electric tests, particularly chronaxia determinations, give the most accurate picture of the extent of paralysis, they are impractical when a great number of patients must be tested, each one with many muscles involved, and with the state of paralysis changing considerably during the first 6 months (8).

There seems to be general agreement, however, that good, comparative results may be obtained simply by testing the active motility and strength of muscles by the old, subjective method of Lovett, about which Herz and Putnam (4b) comment as follows:

> "The patient is first asked to demonstrate his ability to produce voluntary movements; then the power of contraction is tested by movements against resistance exerted by the examiner. Exact quantitative measurement may be accomplished with a dynamometer, but the subjective impression of the investigator is usually sufficient."

In 1945, The National Foundation for Infantile Paralysis (9) recommended terms and standards for recording muscular strength, as determined by resistance tests with and against gravity. These are:

5 is normal (N), no apparent deficiency.
4 is good (G), approximates normal, but fatigues more readily.
3 is fair (F), where part can perform function against gravity but is definitely weak.
2 is poor (P), where muscle is so weakened that it cannot perform its function against gravity but with removal of gravity can function.
1 is trace (T), where there is slight contractility of the muscle.
0 is zero, no evidence of contractility of muscle fibers.

PHARMACOLOGIC TESTS

PROSTIGMINE TEST

Acetylcholine is regarded as the chemical mediator in the transmission of nerve impulses across the myoneural junction. In myasthenia gravis there is some interference with this transmission, with the result that the involved muscles have a higher than normal threshold to the effect of nervous impulses. The characteristic reactions of such myasthenic muscles to electric stimuli have already been discussed (page 388). It is known that physostigmine, and its derivative, prostigmine, counteract the factors inhibiting acetylcholine action and restore the normal neuromuscular performance.

The nature of the interfering mechanism is not yet definitely understood. Bennet and Cash (11) believe the following factors may be involved: deficiency of acetylcholine production, increase in muscular choline esterase, and the existence of some abnormal metabolite with a curare-like action.

The effect of acetylcholine on myasthenic muscles was first observed by Walker (10).

PROCEDURE

The method described is that of Viets and Schwab (12). The patient is examined for those signs of muscular weakness which can be most readily observed, for example, degree of ptosis, ease in talking or swallowing, or strength of the facial muscles.

Prostigmine, in the form of neostigmine, to which is added $^1/_{100}$ grain of atropine sulfate, is injected intramuscularly, and time of injection is noted. The dose for adults is 3 cc. of the commercially available 1:2,000 solution. As undesirable reactions may be elicited in young persons, by such a dosage, the dose for children should be only 1 or 2 cc., as originally recommended by Viets and Schwab even for adults. The amount of atropine need not be changed, since young persons tolerate this drug well.

TABLE 62A
Prostigmine Test. Scoring of Sample Case

Time, minutes	Objective improvement, score	Subjective improvement, score
10	1	0
20	2	1
30	3	2
40	3	3
50	4	4
60	4	4
Totals	17	14

According to Viets and Schwab (12).
The total score is 31—presumptive evidence for the diagnosis of myasthenia gravis.

For 1 hour following the injection the muscle groups mentioned above are closely observed, and the degree of objective improvement is noted at 10 minute intervals as follows:

0: No improvement
1: Slight improvement
2: Moderate improvement
3: Considerable improvement
4: Complete or marked improvement

The patient's subjective feeling of improvement (general feeling of well-being) is scored the same way, and at the same intervals. The total score is obtained by adding the two columns. The maximum score is 48. The scoring of a sample case is given in Table 62A.

INTERPRETATION

Total scores observed in myasthenia gravis vary between 18 and 48. Scores between 8 and 18 are considered doubtful; scores below 8 represent normal (negative) results. In the majority of cases of myasthenia gravis, the effect of prostigmine is so obvious that such quantitative evaluation of the results is unnecessary. But in the early stages of the disease, and in doubtful cases, the scoring scheme is a valuable aid.

For other diagnostic tests for myasthenia gravis, see pages 175 and 388.

QUININE TEST

Quinine affects the neuromuscular junction in a manner exactly opposite to that of prostigmine (page 390). In myasthenia gravis quinine further impedes, according to Wolf and Kennedy (13,14), the already impaired transmission of nerve impulses in myasthenic muscles. In myotonia, on the other hand, in which the nerve stimuli travel too fast and without normal inhibition, quinine partly restores the normal mechanism of transmission. Since myasthenia and myotonia are of mutually opposed natures, there is a corresponding contrast in the changes resulting in these conditions from administration of prostigmine and quinine. Prostigmine encourages restoraation of normal muscular reaction in myasthenic patients; quinine induces partial solution or disappearance of the myotonic manifestations in Thomsen's disease (14).

PROCEDURE

The test, as evolved by Harvey and Whitehill (15), starts with an accurate observation of the functional behavior of the muscles, i.e., the intensity of abnormal, clinical features. The patient is then given 10 grains (0.6 Gm.) of quinine sulfate by mouth, in 2 doses, 3 hours apart. The effects are observed 1 hour after the second dose, and the conditions before and after the medication are compared. The same method of scoring as in the prostigmine test may be used.

INTERPRETATION

A definite lessening of hypertonic muscular manifestations after quinine administration supports the diagnosis of myotonia, while in suspected cases of myasthenia the quinine may aggravate the myasthenic signs or elicit heretofore latent muscular changes. Muscular disturbances not related to either of the two diseases will remain almost unaffected.

CURARE TEST

The test proposed by Bennet and Cash (11) is based on the fact that curare has a very much stronger paralyzing effect on the neuromuscular junctions than does quinine. The abnormalities of electric response in the myasthenic and curarized muscle are identical. Myasthenic conditions are made acutely worse by intravenous administration of small doses of curare. Sometimes a state of paralysis results, requiring immediate injection of the antidote, prostigmine. In view of the inherent dangers of this test method and the lack, so far, of studies on the correct dosage for children, this method cannot as yet be recommended.

PITRESSIN HYDRATION TEST

When drinking of water is forced, and its excretion is prevented by parenteral administration of pitressin, a state of hydration, and probably of intracellular hydration in the central nervous system, results. Such forced hydration is without clinical consequences in healthy subjects. In patients with idiopathic epilepsy, however, the test procedure very often causes epileptic seizures, although water or pitressin alone does not cause such seizures (16). Certain observations point to the probability that forced hydration leads to alkalosis, which stimulates cell permeability and thus precipitates convulsions. Conversely, those factors which are thought to decrease cell permeability, such as acidosis (ketosis) and dehydration, lessen the likelihood of epileptic seizures.

PEDIATRIC CONSIDERATIONS

The pitressin test was originally studied in children but its use in children has now been largely abandoned, and replaced by electronencephalography. The main risks in the test are unexpected pitressin reactions (extreme pallor, dizziness, collapse), and signs of water intoxication (abdominal cramps, excessive vomiting). The technical difficulties of repeated administration of large amounts of fluid and collection of urine samples at specified times further limit the usefulness of this test in children. Nevertheless, if facilities for electroencephalography are not available, the test may supply valuable information, provided it is possible to adhere strictly to the test procedure.

PROCEDURE

The method described is that of McQuarrie and Peeler (16). The child is kept in bed during the test period, and is given the ordinary diet for its age, with a minimum salt intake. At the start of the test, an enema is given, urine is passed, and the patient is weighed. Then 0.1 to 0.2 cc. of pitressin is injected subcutaneously; thereafter, 0.2 to 0.4 cc. of pitressin is injected at 4 hour intervals until 10 doses have been given or until the test is stopped for any one of the reasons listed below. The smallest doses to promote effective antidiuresis should be chosen. Distilled or tap water, 2 to 5 cc. per kilogram of body weight per hour, is given by mouth with the first dose of pitressin and at 4 hour intervals thereafter.

The total food for the 24 hours is divided into 4 equal portions that should be consumed within 2 hours after each weighing.

It has been suggested that the intervals between the administration of the pitressin and water also be prolonged to 6 hours if the procedure cannot be adhered to otherwise.

The test must be stopped immediately if (*1*) an epileptic seizure occurs, or (*2*) fairly marked signs of overdosage become manifest. These include abdominal cramps, excessive vomiting, irregular pulse, extreme pallor of skin and mucous membranes, weakness, dizziness, unconsciousness, and collapse (17).

A complete record of the progress of the test must be kept, including the time and amount of food intake, time and amount of water and pitressin given, weight, and volume of urine voided.

The test is valid only if a positive water balance is induced, i.e., an increase of at least 2.5 per cent in weight, as compared to initial weight.

The test is contraindicated for children with impaired cardiac or renal function.

INTERPRETATION

Retention of water takes place in both normal and epileptic children. But if seizures occur in the course of the test, there is a fair probability that the patient is suffering from idiopathic epilepsy.

Absence of convulsions with effective hydration is considered a negative test result, and speaks against a diagnosis of epilepsy.

Hilger, Mueller, and Freed (18) have given a good description of the test as used in adults.

CREATINURIA AND MUSCULAR DISEASE

A discussion of the significance of creatine and creatinine excretion, both normal and abnormal, will be found on pages 173–177. Discussion here will therefore be confined to the place of creatine and creatinine coefficients in the diagnosis of muscular disease. Peters and Van Slyke (19) state:

> "Spontaneous creatinuria accompanies almost all conditions attended by atrophy or extreme functional disorders of the skeletal musculature. It has been observed in myasthenia gravis, amyotonia congenita, myotonia atrophica and muscular wasting from a variety of causes; in generalized myositis fibrosa, anterior poliomyelitis, congenital muscular hypertrophy, amyotrophic lateral sclerosis and diffuse myositis."

By far the greatest quantities of creatine are commonly excreted by patients afflicted with the pseudohypertrophic type of progressive muscular dystrophy. Whenever there is a considerable creatinuria associated with these muscular disorders, the urinary creatinine tends to decrease, but not enough to keep the total creatinine coefficient from rising above normal.

In addition to the excessive creatinuria, such muscular disorders, particularly the progressive dystrophies, show a decreased tolerance to loading doses of creatine, an intolerance that can be exaggerated by ingestion of glycine (20).

Analogous disturbances of creatine metabolism have been revealed by Shorr, Richardson, and Wolff in hyperthyroid patients (page 448). The nature of the muscular weakness in Graves' disease is considered to be similar to that of progressive muscular dystrophy, but only in the former condition will iodine correct the impairment of muscular phosphocreatine metabolism.

It is assumed that a diminished creatinine output in muscular disease reflects an accompanying decrease of actively functioning muscular tissue, i.e., muscular wasting. Creatinuria is normally related to the subject's age (p. 173 ff.). Creatine excretion in excess of normal, in relation to age, indicates deficient function of some part or parts of the active muscular mass (21). However, the evidence of impaired utilization of creatine fails to show the site and nature of the underlying metabolic disturbance.

ELECTROENCEPHALOGRAPHY

Electric potentials originating in the brain are recorded by means of electrodes placed upon the scalp. The tracing thus obtained is called the electroencephalogram (EEG); it shows waves of varying duration and voltage. Records from symmetric points of the hemispheres are almost identical, while records from different regions, e.g., the frontal and occipital, show definitely different patterns. In the occipital region the pattern is dominated by the alpha waves, which in adults have a frequency of 8 to 12 cycles per second, or a duration of $^1/_8$ to $^1/_{12}$ second per wave. The voltage of these alpha waves ranges between 10 and 100 microvolts. Less alpha and more beta activity appears in the anterior regions of the hemispheres. The beta waves show frequencies of 15 to 30 cycles per second, and a lower voltage than the alpha waves. Lower frequencies than those of the alpha waves have been called delta waves; in a normal adult they appear only in records made during sleep.

Abnormalities in the electroencephalogram consist of greater bilateral differences than those found normally, and in the appearance of abnormal waves or patterns from one or more electrodes.

Diagnostic interpretation of the electroencephalogram should be left to specialists in this field.

PEDIATRIC CONSIDERATIONS

Electroencephalography is feasible in children, whatever their age. While the actual procedure must be left to one with special training, the pediatrician must know the general principles of the method if he is to follow the specialist's description and conclusions. Furthermore, he should be familiar enough with the technical procedure to be able to anticipate the difficulties in obtaining an electroencephalogram in a child of a given age. And finally, familiarity with those clinical problems in children in which the encephalogram may be a diagnostic aid is of the utmost importance.

It is with these pediatric requirements in mind that the following brief survey of the process is given.

PROCEDURE

According to Strauss (22a), the equipment consists of *(1)* electrodes* which pick up the potentials from the scalp; *(2)* amplifiers which amplify these potentials sufficiently to activate the writing system; *(3)* ink writing recorders which trace the potentials on paper moving past the pen. Besides the stationary instruments, reliable portable machines have been developed recently which permit taking an electroencephalogram wherever alternating current is available.

The number of electrodes used depends on the diagnostic problem presented. Two electrodes may suffice to diagnose the presence or absence of epilepsy, while 10–15 electrodes are necessary to diagnose localized brain lesions. As a rule, the electrodes are fastened to the scalp with collodion, but in young babies with very little hair they may simply be held in place with adhesive tape. Though the application of the electrodes is entirely painless, younger children will not always hold still. Their resistance must be overcome by keeping the heads in a fixed position for as long as it takes to apply the electrodes, about 10 to 15 minutes for a whole set.

The recording itself takes 15 to 20 minutes. During this period the patient should lie motionless, with eyes closed. These standard conditions usually cannot be attained in children up to 4 or 5 years of age. It is a difficult task indeed to induce a 2 year old to lie on a couch motionless and with closed eyes. However, by having the child sit on a chair or on somebody's lap, it is possible to obtain at least short periods of relaxation and rest even in intractable children. Even if the child moves during the recording period, the tracings can be utilized. Such movements appear as artefacts in the record, and are easily distinguished from the valid parts by the experienced observer.

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* As a rule, solder buttons are used, but needle electrodes may be needed in very restless children.

To induce young children to keep their eyes closed is an almost hopeless task. While this affects the frequency of the waves throughout the whole record and makes it impossible to judge the maturity of brain function in a given child, it does not interfere with the diagnosis of epilepsy and organic brain lesions.

Administration of sedatives should be discontinued 48 hours before the recording. Such drugs, while making a child more tractable, may induce some diffuse changes in the tracing. However, recent observations by Gibbs and Gibbs (22b) seem to indicate that induction of sleep can also constitute an aid to electroencephalographic diagnosis, particularly of localized lesions and of epilepsy. To obtain such "sleep records" a single dose of seconal is given the patient before applying the electrodes, $^3/_8$ grain to infants, $^3/_4$ grain to children (22c).

INTERPRETATION

In normal children, the pattern of the electroencephalogram changes gradually from birth to about the age of 15 years. The newborn child shows no well-defined rhythm at all; definite ryhthmic activity first appears at about 3 months. The basic pattern at this time is formed by waves with a frequency of 3 to 4 cycles per second and a voltage as high as 100 microvolts (average 60–70).

TABLE 62B
Average Values for Occipital Alpha Frequency in Normal Children

Age, years	Alpha waves, per second	Age, years	Alpha waves, per second	Age, years	Alpha waves, per second
1	4.5	6	8.0	11	9.5
2	6.5	7	8.5	12	9.5
3	7.0	8	8.5	13	9.0
4	7.5	9	9.0	14	9.0
5	7.5	10	8.5	15	10.0

From Lindsley (23).

The frequency of the waves then increases gradually up to the age of 14, while the voltage tends to decrease. The adult frequency is reached at about the age of 15. Average values for the occipital alpha frequency for the ages from 1 to 15 years, as given by Lindsley (23), will be found in Table 62B. These values, however, are average values; the variations at a given age are great. Except when deviations from normal are very marked, it is difficult to decide from the tracing whether the general cerebral development of a child is retarded or not. Figure 41 shows typical records of 2 normal children, 4 and 9 years old.

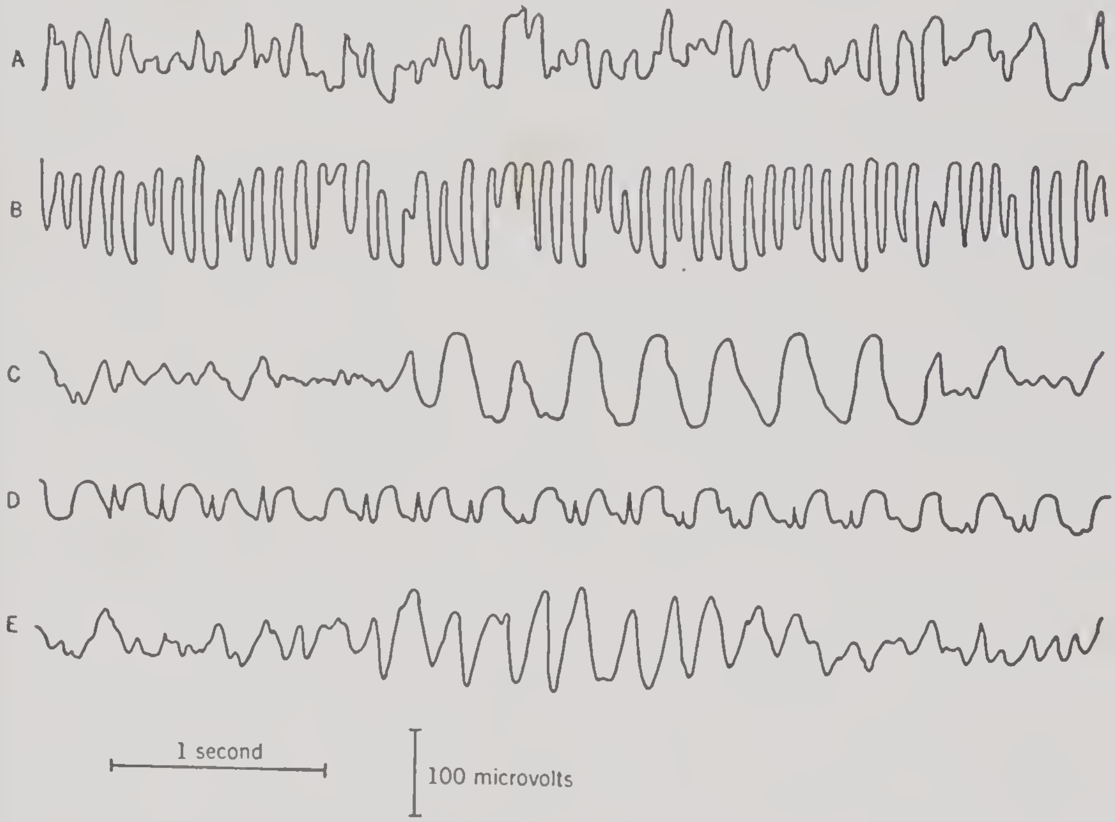

Fig. 41. Normal and abnormal electroencephalograms. A: 4 year old child, no pathology. B: 9 year old child, no pathology. C: 9½ year old child, petit mal epilepsy; record made between attacks; bursts of high voltage slow activity. D: 10 year old child, petit mal epilepsy; record made during hyperventilation; spike–wave pattern. E: 16 year old child, behavior problem; bursts of high voltage slow activity. Courtesy of Dr. H. Strauss, New York City.

Abnormal patterns are obtained in many disorders of children. The outstanding pathologic change is the appearance of abnormally slow waves. Such delta waves may be obtained from all electrodes, from only one, or from several electrodes, depending on whether the underlying pathology is focal or diffuse in character.

The electroencephalogram has been particularly valuable in diagnosing epilepsy. Changes in the tracings associated with epileptic disorders are characterized by the paroxysmal appearance of abnormal potentials, such as (*1*) high voltage slow waves (usually 3–6 cycles per second); (*2*) high voltage fast activity (spikes, with an approximate frequency of 30 per second); and (*3*) composite patterns, such as the spike–wave pattern, appearing usually with a frequency of close to 3 cycles per second.

Jasper (24) holds that none of these types of records are pathognomonic for any of the main clinical types of idiopathic epilepsy (grand mal, petit mal, psychomotor type). However, the most common abnormality found *between attacks* in any form of epilepsy are bursts of high voltage slow waves (Fig. 41C), whereas bursts of spike–wave pattern (Fig. 41D) are always found during a petit mal attack. Sometimes they are also found between attacks in patients with petit mal, as well as in other forms of epilepsy.

If such pathologic patterns are obtained diffusely over all parts of the brain or from symmetric left and right regions, idiopathic epilepsy is suggested. The appearance of such potentials from one electrode, or from one side only, is indicative of symptomatic epilepsy and focal pathology. Bursts of high voltage slow waves have also been found in nonepileptic children with serious behavior and conduct disorders (25), termed by Jasper (24) "ictal automatism of behavior." Such a tracing is reproduced in Figure 41E.

Electroencephalogram after Hyperventilation

It has long been known that hyperventilation may induce epileptic attacks in adult epileptics. This is equally true for children. A tracing which is normal at rest may show changes characteristic of epilepsy when taken during the breathing process.

PEDIATRIC CONSIDERATIONS

Hyperventilation is possible with children over the age of 4 years. In a large percentage of normal children hyperventilation produces random slow waves of high voltage, which have no diagnostic significance. The younger the child, the more pronounced are these changes. According to Brill (25), positive reactions occur in approximately 40 per cent of all children between the ages of 4 and 6, in 30 per cent between the ages of 6 and 8, in 20 per cent between the ages of 8 and 10, in 8 per cent between the ages of 10 and 12, and in 18 per cent between the ages of 12 and 15. However, the appearance of the spike–wave pattern or of well-defined bursts of slow activity during hyperventilation has the same diagnostic value as in adults.

PROCEDURE

The electroencephalogram is first taken with the child at rest. Then the child is asked to breathe deeply through the mouth, and, particularly, to exhale completely. As a rule, this is carried on for 2 minutes while the recording is continued.

INTERPRETATION

Only the presence of the spike–wave pattern, or of well-defined bursts of slow activity, can be considered indicative of epilepsy. Random slow activity, as mentioned above, has no diagnostic significance in children.

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CHAPTER XIII

Psychologic Tests

BY KATE L. KOGAN, PH.D.

The psychologic test is an instrument designed to measure a single trait, ability, or power, or a cluster of such traits. The individuals who make up our social world are different one from the other, and we must have some means of recognizing and evaluating these differences. We tend to refer to people as "bright" or "stupid" as readily as we refer to them as "tall" or "fat"; we must, therefore, rely on a system of measurement to check our subjective judgment.

Most psychologic tests are technics enabling one to observe the child objectively in a standardized situation, that is, to set him up against a yardstick, and to compare his reactions directly with those of other children. In other words, instead of each person judging on the basis of his own experience alone, each judge is provided with the same extensive basis of experience.

TYPES OF PSYCHOLOGIC TESTS

ABILITIES MEASURED

Psychologic tests can be classified in several different ways. In the first place, they can be grouped according to the mental or behavior traits which they measure. There are the tests designed to measure *intelligence,* most simply defined as learning ability. Ideally, these tests must measure intellectual growth uncontaminated by other factors. The material must be equally familiar to all children, so that we measure innate ability to deal with the material and fulfill the test requirements rather than individual differences of background and experience. Test material must confront the subject with situations in which all children of that age have presumably had equivalent experience. This can be accomplished in either of two ways. Tests can deal with situations to which all children have been exposed a great deal, for example, vocabulary, building sentences, naming colors, counting, or describing what they see in pictures; frequent contacts with these activities accompanied by failure to learn from these experiences presuppose absence of learning ability. Some tests, however, deal with test materials with which no child has had special opportunity for practice. Here are the construction–puzzle type tests, or the tests requiring repetition of certain sentences or number series. In this way, "other things being equal," differences in test successes are due to differences in native capacity or endowment. In this chapter, the greatest emphasis is placed on tests of intelligence, as being the most important single group of tests; other types of examination will be discussed more briefly.

In direct contrast, there is a group of tests which measure *achievement*—the amount of specific knowledge already acquired—rather than the potential ability to acquire knowledge, as in tests of intelligence. These may be tests of educational subjects (reading, arithmetic, spelling), vocational skill (clerical work, mechanical ability), or special talent (drawing ability).

Another group of tests evaluates emotional and personality traits by showing the child's preferences, interests, feelings, or worries. These are usually known as *personality* tests. They frequently refer both to the child's own feelings and to his interaction with other people, so that they measure social adjustment as well.

FORMS OF TEST MATERIAL

Tests can also be divided according to the formal aspects of the test material. Two general groups are the *verbal* tests and the *performance* tests. In the verbal test, the child is asked questions to which he responds, or is given verbal instructions which he follows. The verbal test may measure intelligence, achievement, or personality. The performance test may also measure any kind of trait or ability. In the performance test the child deals with concrete objects, so that he does something, or tells something about an actual object, rather than says something in response to a question.

There is a special group of tests, falling under the heading of performance tests, which employs a rather unique methodology for the evaluation of personality. Children, especially younger ones, have difficulty expressing their feelings and are unable to talk fluently about their likes, worries, or hopes, with the result that tests which ask questions yield somewhat limited information. Another type of examination has therefore been devised. The child is faced with a situation which has within itself little or no definite meaning, so that in making his interpretation of it he must draw upon his own particular ideas or experiences. These methods are known as *projective technics*, since the child projects his personality and attitudes into the situation in order to give it meaning for him. This is true of all test responses to a certain extent, but these particular technics exaggerate the projective factor.

The best known of the projective technics is the Rorschach or ink blot test (1,2,8) in which the child tells what the formless ink blots "look like to him." Space does not permit detailed discussion of the method. Suffice it to say that it is widely used in most schools, courts, clinics, hospitals, and agencies dealing with children, their families, and their problems.

Puppets, paints, pictures, or clay can all serve as a medium through which the child can project his problems. Other projective methods are equally helpful in certain situations.

METHODS OF ADMINISTRATION

Tests can also be divided into *group* tests and *individual* tests. Group tests are widely used for selection, classification, or screening purposes—wherever the problem requires planning for a great number of people. They provide a quantitative score by which the person is compared with others of his group. Group tests have little or no place in a clinical study. To understand the individual, we examine him apart from the group. Clinically, it is important to learn more than merely whether the child can or cannot do a certain task correctly. It is necessary to know how he arrives at his responses, the nature of his learning and reasoning processes, and his emotional reactions to different situations. Frequently, the kind of error he makes is more significant than the fact of success or failure. One can then evaluate the process rather than only the result.

MEASUREMENT OF INTELLIGENCE

RATIONALE OF TEST CONSTRUCTION

The earliest and best-known standardized instrument for measuring intelligence was devised shortly after 1900 in response to the plea of the Paris schools that they must be able to identify and isolate feebleminded children in order to avoid unnecessary expenditure in unsuccessful attempts to educate them. The measure which was formulated was the Binet-Simon test. It was soon adapted for use in this country by Dr. Lewis Terman at Stanford University, and resulted in 1916 in the Stanford-Binet Examination (10), and in the Revised Stanford-Binet Examination, developed in 1937 by Drs. Terman and Maud Merrill (11). They are the most widely used measures of intelligence today.

Binet and Simon, and later Terman and his colleagues, attacked their problem in the following way. A number of tasks were given to large, randomly selected, groups of children of different ages. These tasks were then grouped in terms of the critical age level at which they measured success. Binet's decision was that if 60 to 90 per cent of children of a given age group could pass a test, the test was suitable for that age. If *all* children (all 8 year olds, for example) could succeed with a test, it was too easy to be a measure of 8 year ability, since the unselected standardization group was assumed to include some retarded 8 year olds. On the other hand, if only half the group could succeed, the test was not representative of 8 year ability, but only of bright or exceptional 8 year olds. By this means, an age-scale was constructed with selected items known to be representative of the abilities of children at each age level. It was also necessary for the items to discriminate clearly between adjacent age levels.

UNITS OF MEASUREMENT

If the tests and questions of the age-scale are now given to a specific child, he can be rated in terms of the age level at which he can succeed with the tests. In other words *mental age* (M.A.) represents the ability of the child by indicating the average age at which comparable ability is normally exhibited. The child who can succeed with the tests at the 8 year age level has a mental age of 8 years, or does as well as the average 8 year old. Thus, mental age furnishes a measure of the amount of mental ability which is present at the time of examination.

For predicting a child's mental abilities at a later age, however, we need the Intelligence Quotient (I.Q.), i.e., a measure of the rate of mental development. The empiric basis of the quotient's conception is the observation that a slow child usually remains slow, and that a quick learner generally maintains rapid learning and development. The I.Q. is computed as follows: If a given 4 year old is backward in development, having reached a developmental level of only 2 years, (M.A., 2 years), the ratio of his present mental development to his actual age (mental age divided by chronological age), is 0.50. Thus we assign him an I.Q. of 50 (dropping the decimal point), indicating that his mental development is only half as rapid as that of the average child. We can also predict that at age 8 he will be mentally near the 4 year standard, and that at the cessation of mental development, at 15 to 16 years of age, he will have the ability to succeed with tasks of about the 8 year old level.

A few tests express scores in other terms, such as percentile rank. For example, a task may have been given to large groups at each age, as above. Scores for a given age group will then be arranged according to percentage distribution, e.g., the highest 10 per cent, the next highest 10 per cent, and so on. An individual child's score can then be expressed in terms of its percentile rank in an unselected group of children his age, the percentile score being the point on the scale below which fall a given percentage of unselected cases: an individual in the 90th percentile is one who is exceeded by only 10 per cent of the test population.

DISTRIBUTION OF INTELLIGENCE

As with other human traits, intelligence ratings in the general population are distributed according to the "normal" or "probability" distribution. There are a few individuals at the upper or lower extremes, and there are many people rated at or near the average. I.Q.'s are grouped around 100 as the central point, with a sharply declining curve toward upper and lower limits. Although there are no clearcut or absolute points of division, the following groups can be separated for purposes of convenient description. It must be remembered, however, that the measuring scale is a continuum, and that there is no real gap between the upper limit of one group and the beginning of the next higher classification.

Mental Deficiency. The group with I.Q. below 70 constitutes 1 per cent of the population. The *idiot,* with an I.Q. below 25, never reaches a level of more than about 3½ years. The *imbecile,* with an I.Q. between 25 and 50, reaches mental levels up to 7½ years at maturity. These two groups require close supervision and special custodial care. They are usually cared for in institutions, or need comparable constant care at home. They can never be responsible for their own safety and cannot learn much useful work. At the lower levels they must be helped in caring for all personal needs. Physical development is frequently correspondingly retarded. The *moron,* with an I.Q. between 50 and 69, may finally reach a mental level of around 10 years. They, too, are frequently cared for in institutions, but can profit by a training program in which they learn simple housework, sewing, farming, or one-operation industrial procedures. Their work needs a great deal of supervision, and they cannot be expected to exercise judgment or to change to a procedure other than the one they have been taught. They need close supervision in matters relating to money, recreation, and social activities, in order to avoid delinquency; sex delinquency is especially frequent in girls; they are easily influenced by unscrupulous people, as they do not have the mental ability to foresee the consequences of their acts. Many of these children are to be found in the community; they can adjust in "special" or ungraded classes in school, where they learn a few of the fundamentals of the academic "tool subjects," much handwork, and practical work assignments.

Borderline Intelligence. The group with an I.Q. of 70 to 79 constitutes about 5 per cent of the population. These children reach a mental level of 11 to 12 years. They progress best in special classes and usually attain an academic level of the fourth or fifth grade, thus obtaining the essentials for simple, independent living. They can be self-supporting in routine unskilled labor jobs. They may or may not become social problems depending on their emotional adjustment and on their acceptance in the community.

Dull Normal Intelligence. The group with an I.Q. of 80 to 89 constitutes about 14 per cent of the population. These children are in regular school grades, but usually repeat two or three grades, perhaps completing the eighth or ninth grade by the time they have reached the age at which they may leave school. Since the regular curriculum, as well as camp, playgroup, and other activities, is geared to the average child, they are handicapped in competition with brighter children and are repeatedly faced with failure. Often there are average siblings in the family with whom they are compared to their disadvantage. As a result, behavior and personality problems are particularly frequent in this group. Often they resort to excuses of physical ailment and weakness to explain their deficiencies.

Average Intelligence. The group with an I.Q. of 90 to 109 constituting 60 per cent of the population, is the one for whom society is planned. This does not mean that these children have fewer problems or difficulties, but their intellectual ability is not often a primary factor contributing to their difficulties. Barring unusual circumstances, they should complete two to four years of high school without difficulty, and become productively useful citizens.

Bright Normal Intelligence. The group with an I.Q. of 110 to 119 comprise about 14 per cent of the population. These children progress a little more rapidly than the average. They may skip one or more grades. They do well in academic high school courses and may take some technical training on a higher level. In this group are found salespeople, office workers, and many of the white collar employees.

Superior Intelligence. Children with an I.Q. above 120, comprising about 6 per cent of the population, learn quickly, and their rapid development usually becomes apparent even in early childhood. They walk and talk at an early age, and physical development is frequently accelerated. There is usually no school difficulty, and choice of occupation or vocational training depends more upon specific talents and personality attributes than on learning ability. Most of these children are good college material, or can take specialized training of equal levels of difficulty. In childhood, however, these children may have difficulty in adjusting. They are misfits in the sense that they cannot compete physically and socially with children who have the same mental age, and they may be removed in interests and abilities from those of their own size, strength, and emotional maturity. Personality problems and emotional conflicts frequently occur, although their good intelligence helps them to work out many of their problems. Moreover, they are likely to associate with other children of similar I.Q. in school and in social life, so that problems of adjustment are minimized.

CONSTANCY OF THE I.Q.

Unlike other laboratory methods and procedures, many mental tests cannot be repeated frequently with reliable results. The scientist can repeat his laboratory experiment under identical conditions, to verify the accuracy of his results. The child, having experienced one psychologic examination which he can recall clearly can never experience another as though it were for the first time; identical conditions cannot be achieved. Although some tests have alternate or parallel forms, the desirable interval between one test and another for meaningful results is usually about one year. The effect of practice is difficult to evaluate, and differs both from one child to another, and from one type of test to another. Some tests requiring factual answers are definitely invalidated by repetition; others, such as memory span, are less subject to improvement by repeated performance. Some children are bored by familiar material, only half listen to instructions, and make impulsive errors, doing less well on their second trial; others are spurred to improve upon their earlier performance. Personality tests and tests of emotional adjustment often constitute an exception to this principle. These tests can safely and often very fruitfully be repeated at intervals to gage the extent to which change and adjustment are taking place.

Children of school age show retest scores of remarkable constancy over fairly long intervals. In most cases, variability in I.Q. is 5 points or less, and only in rare cases is it more than 10 points. While changes in environmental opportunity may raise the I.Q. slightly, or lack of normal growth opportunity may lower it, the range of change is not usually wide. Cultural deprivation, physical illness, or emotional disturbance may interfere with mental growth. It is unusual for a child's rating to change more than 10 points as a result of the influence of one or more of these factors.

EFFECT OF AGE ON TESTING INTELLIGENCE

When the child does not yet use speech with sufficient facility to express his ideas, intellectual development cannot be evaluated apart from physical development and maturation. Mental growth in infants and young children is therefore judged by comparison of their spontaneous behavior with developmental standards. As an illustrative method, one may cite the system devised by Dr. Arnold Gesell and his co-workers (5). It is based on carefully assembled schedules of child development, covering the first 5 years of life. These standards are defined in terms of ability to perform specific acts with reference to motor development, vocalization, manipulation of objects, and responsive awareness of other people.

From about the age of 2 years until the age of entering school the child is able to understand directions and respond to test material as he is instructed, so that more formal testing is possible. However, many other factors may still interfere with completely reliable testing. The development of speech, motor skills, or manipulative dexterity may be retarded for other reasons than limited intelligence, yet the retardation will impose a handicap in performing the tests. The preschool child is likely to be shy in the presence of strangers, fearful about being "deserted" by the mother in the examining room, timid about speaking to the examiner, and unaccustomed to following directions given by someone other than the parents.

TABLE 63

Age Range of Five Selected Psychologic Test Methods

Test method	Age, years																	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Adult
Gesell Developmental Schedules	←	—	—	—	—	→												
Minnesota Preschool Scale		←	—	—	—	→												
Revised Stanford-Binet			←	—	—	—	—	—	—	—	—	—	—	—	—	—	—	→
Cornell-Coxe Performance Ability Scale						←	—	—	—	—	—	—	—	—	→			
Wechsler Bellevue Test											←	—	—	—	—	—	—	→

Constancy of the I.Q. is less clearly demonstrated in infant tests and examination of young preschool children. This is not because the development of these children is necessarily less even, but because rating methods and the criteria of observed behavior by which mental growth is estimated are probably less reliable indices. It is therefore considered valid merely to classify the child below school age as defective, retarded, average, or advanced.

Table 63 indicates the age range of a few selected tests which together cover the childhood period. It is more effective to select tests which extend well above and below the child's age standard, rather than one which applies only its upper or lower limits. For example, a 5 year old is less well examined by a preschool tests whose upper limit is 5 years; other tests permit him to exhibit some 6 or 7 year old abilities if he possesses them. For this reason all tests are more informative in their middle ranges.

SELECTION AND APPLICATION OF PSYCHOLOGIC TESTS

GENERAL PRACTICAL CONSIDERATIONS

In the clinical study of a child referred to the psychologist for examination, a number of problems should be given consideration. The procedure is flexible, and must be adapted to the immediate situation. Which tests shall be combined into a battery and the specific points of investigation will be determined by the perspicacity and experience of the trained clinician, the clarity with which the child's reaction pattern is revealed in the test situation, and the nature of the problem for which examination is being made. It is important to stress that the referring physician, requesting psychologic examination of a patient, will find the test results more useful if he can delineate the nature of the problem and the pertinent history beforehand. What is the immediate situation, and what adjustment is desired? What are the troublesome symptoms, either physical or behavioral? What are the high points of the past developmental and health history?

The lines along which a psychologic examination will be conducted are decided as the tests progress. Tests given early in the process serve to give a broad general view of the child's abilities, an over-all picture. One might symbolize it as a study through a "low power lens." Then, as distinctive features appear in the child's performance, we return to examine them more closely and more intensively. At the points at which the child deviates from the expected standard, revealing either exceptional skill or notably poor performance, we study his abilities through a "high power lens." Therefore, the first point of investigation will probably be the administration of one or more tests of general intelligence, including verbal and performance items. How does the child compare with others of his age? Is slow learning ability indicated? This helps to sketch in the general picture; we now look to details. Does his performance seem to be the result of native endowment, or is there unevenness of abilities which are suggestive of a special defect? Might the defect be sensory in nature—a disturbance of hearing or vision—or are there characteristics (e.g., slowed reaction, impulsiveness, short attention span, poor immediate memory) which are suggestive of disturbance of the central nervous system, and does the medical history describe the possibility of such impairment? Does the pattern of test scores conform with that usually exhibited in some specific neurologic disorder? Tests are not to be considered diagnostic of the physical condition, but diagnostic of the mental function which characteristically accompanies somatic pathology.

Since intelligence tests are usually based on the assumption that intelligence is a composite made up of many different kinds of abilities, and since the tests therefore consist of a variety of questions, we must investigate how well the child does with different types of test material. What are his ratings on verbal tests? This is the kind of test score which has the highest correlation with school success, and therefore gives the best prediction of academic progress. How well does he do with constructive or manipulative tasks in the performance tests? In either case, what can be said of his planning ability, memory, persistence in the face of difficulty, or ability to refer experience from one situation to a similar one? Many times children do well with one type of material and poorly with another. Some people are facile in verbal expression and can describe clearly what they mean. Others can think more clearly when they can see and handle the situation in concrete form. Many retarded children deal much better with concrete material. Certain neurotics have a good stock of familiar, well-learned factual information but do poorly with new situations requiring logical analysis, or with social situations bordering on their own discomforts. Certain organic pathologies cause specific impairment of visual space-form perception, resulting in wide discrepancies between verbal and performance test scores. Other pathologies are accompanied by difficulty in abstraction or generalization, and these patients can deal effectively only with concrete situations.

If at this point we have evidence of a handicap (lameness, spasticity, blindness, deafness, cardiac disease), with certain specific areas of impairment, we must find out how severely the handicaps limit him. What are the child's best abilities? How can these be encouraged and developed in order to help him achieve a rich and useful life and compete successfully with others? Moreover, what is his emotional attitude toward his handicap, and can he be helped to develop certain personality attributes which will help him to live more comfortably with others?

In the light of all the preceding information, how well is the child's environment suited to him? Can he achieve at the level expected and set up for him—be it in school, with piano lessons, with household chores, or in social relationships? Special educational achievement tests can tell us about his actual accomplishments. Does he live up to his potential achievement? If he has the capacity for success, but still fails, what is the cause? What are his talents and vocational assets aside from intelligence? Has he any outstanding aptitudes?

When there are problems involving slowed progress or social maladjustment which are not readily explained by restricted endowment or limited training, we must search further. What indications are there of emotional problems? To what extent could physical complaints be the expression of emotional or personality disorder? Are there symptoms such as enuresis, stomachache or headache, feeding problems, or speech difficulties for which no organic basis can be found? Is a particular child, who seems superficially to be merely "placid," a rigid, tense neurotic whose phantasy is filled with so many anxieties that he is afraid to express any emotions? Or is he an emotionally shallow child whose feelings find direct impulsive expression and are thus fleeting? Are social relationships easy for him? Are we justified in placing him in an active play group, or is this the kind of situation he will use any sort of complaint or excuse to avoid? Does he feel secure in family relationships, or does jealousy, rivalry, or insecurity force him to exaggerate symptoms to gain attention and affection? Is he naturally or physiologically sluggish, or do his problems of adjustment absorb so great a portion of his energies that there is little left over to be diverted into play and learning activities?

There are various environmental factors and influences which may artificially distort test results, and must be taken into account. Foreign language handicaps, confusion caused by two languages spoken in the home, as well as speech or hearing disturbances, may place a child at a disadvantage in verbal tests. Low grade children brought up in superior surroundings often develop a vocabularly and social manners which lead one to think of them as being brighter than they actually are. They learn superficial things by frequent repetition. Parental attitudes of overprotection leave the child unable to cope with the test demands in the absence of this customary support, since he has never learned to take the initiative, plan for himself, or express an opinion. In very young children, speech is often delayed because an oversolicitous parent or nurse fulfills every wish before the child has to learn to ask for it. Sociocultural deprivation, such as lack of contacts with other children or absence of material things, limits the child's familiarity with test situations, so that the tasks no longer measure learning ability.

It must be reiterated that psychologic tests do not measure mental abilities directly, but reflect them from the child's behavior and responses. Therefore, test results are by no means infallible. If results contradict the general picture, they are to be questioned, unless some reason for the discrepancy can be found. A child never does well "by mistake." If he scores successfully, it is because he has the capacity to do so. But he can make a falsely low score if he is frightened, negativistic, not feeling well, or does not understand what is expected of him.

For adequate examination, a child should be seen on at least two different occasions, since behavior frequently varies according to the situation. Factors totally irrelevant to the examination—fatigue, digestive discomforts, recent disciplinary measures—as well as feelings toward the strangeness of the test situation involving a new person, and new place, and new activities, contribute to behavior variations. The individual test session may last from half an hour to two hours, depending on the capacity of the child to maintain a highly motivated level of effort. Total examination time varies with the test battery.

It has already been stated that in the majority of instances I.Q.'s are relatively constant. However, exceptions do occur. When a psychologic test rates a child at a significantly lower level than he had previously been rated by comparable examination procedures, several factors must be considered. Actual *deterioration*, i.e., loss of abilities once developed, probably occurs only in the presence of organic degeneration or frank psychopathy. However, a sick child or an emotionally disturbed one may reach a point where he fails to assimilate new experiences and information at his usual rate. Mental age will therefore increase slowly, if at all, and with increasing chronologic age the I.Q. will be lower. This does not mean deterioration, as it would in an adult. If the disturbing factors can be removed, the child is likely to resume mental growth at his normal rate.

Finally, it may be well to consider the problems of introducing the test situation to the parent and child, and explaining its results at a later time. The child's preparation for the test procedure presents a problem only in exceptional cases. He should know before coming for the examination that there will be no treatment, medicine, or painful procedure, but that he will be asked to go into a pleasant room alone with the examiner, and will talk and play games there. Test results should be given to parents only in interpretive form, and *scores should never be supplied.* Just as the raw data of a blood count or blood sugar test would be totally meaningless to the parent, test scores are technical information. It is important to explain the findings to the parents in terms of the child's everyday activities and their provisions for him.

TWO PSYCHOLOGIC EXAMINATIONS, ILLUSTRATING TEST MATERIAL AND ADMINISTRATION IN DETAIL

Two "typical" psychologic examinations will be described step by step, with full description and discussion of the tests used, the reasons for their selection, and the significance of the findings.

CASE I

R. was an 11 year old boy referred for psychologic examination in connection with a thorough case study. At the age of 5 he had been hit on the head with a baseball bat; he sustained a scalp wound, but there was no unconsciousness. He had been subject to petit mal epilepsy since the age of 7. Attacks were first noted when he was doing arithmetic homework, and had gradually increased in number as he grew older. Medication initiated about a month before this examination had controlled the attacks with marked success. At the time when he was referred for psychologic examination, the boy was in the fifth grade at school. The diagnosis of the referring physician was "idiopathic epilepsy."

Revised Stanford-Binet Test. There was no record that he had ever received psychologic examination before. The first step.

therefore, was to measure his general mental ability, and he was given the Revised Stanford-Binet examination (11). This scale is the most widely used measure of general intelligence, and covers a range from 2 years through 3 superior adult levels. It is generally a part of every psychologic test battery for children, although other measures standardized on adults are thought by some clinicians to be preferable for patients 14 to 15 years of age and over.

Since R. had been having some difficulty with his lessons, it was suspected that he might be somewhat retarded, and the examination was begun with items which might be well within his capacities; 8 and 9 year items were introduced, continuing with easier questions until a test level was reached at which he succeeded with all items. He passed all 6 year tests: (*1*) defining given words from a vocabulary list, (*2*) reproducing from memory a design made of 7 round and square beads arranged alternately, (*3*) identifying the missing parts of pictures—the wheel of a wagon, a shoelace from a shoe, the handle from a teapot, one ear of a rabbit, one finger of a glove, (*4*) counting 5, 7, and 9 blocks, (*5*) selecting one dissimilar picture from a series of 5 pictures, and (*6*) tracing simple mazes. This credited him with a "basal age" of 6 years. It is assumed that having succeeded with all 6 year tests he could succeed with all items easier than these.

At the 7 year level he failed one of 6 items. The task was to complete sentences calling for opposite analogies: the point of a cane is blunt, the point of a knife is "steel" was his response. The majority of 7 year olds can supply "sharp" in this sentence. He received credit for correct responses to (*1*) recognizing picture absurdities, (*2*) telling how wood and coal, an apple and a peach were alike, (*3*) copying a diamond successfully in 2 out of 3 trials, (*4*) telling the thing to do if he broke something belonging to someone else, and telling what to do if on the way to school he noticed that he was in danger of being late, and (*5*) repeating five digits forward. Since each of the 6 tests receives 2 months' credit, he was credited with 10 months at this level.

At the 8 year level he still met the vocabulary standard (8 words from the list correctly defined). He also answered factual questions based on a story read to him by the examiner, and he recognized the absurdities in certain statements. He was able to tell the likenesses and differences between a baseball and an orange, a kite and an aeroplane, and an ocean and a river. He failed comprehension questions, not knowing what makes a sailboat move, nor what to say if he were in a strange city and someone asked him how to find a certain address. He could repeat neither of two sentences after the examiner: "Fred asked his father to take him to see the clowns in the circus," or "Billy has made a beautiful boat out of wood with his sharp knife." He therefore passed 4 out of 6 tests at this level, and was credited with 8 months.

At the 9 year level he passed only two items. He gave rhymes—a color that rhymes with head, a number that rhymes with tree, and a flower that rhymes with nose. And he was able to repeat 4 digits backward. He was unable to visualize how a folded and cut square of paper would look if it were unfolded, he could not reproduce simple designs which were exposed for 10 seconds, and he could not detect absurdities in more difficult statements. He also said that he did not know how much change he should get if he bought 4 cents' worth of candy and gave the storekeeper 10 cents, nor 12 cents' worth of candy out of 15 cents. He earned 4 months' credit at this level.

At 10 years his vocabulary was still credited (11 words). He identified the absurdity of a pictured situation. He read a selected passage aloud without error in less than the time allowed, and could recall 12 separate ideas from the paragraph. He was able to give 2 reasons why children should not be too noisy in school, but could not give 2 reasons why most people would rather have an automobile than a bicycle. He could name only 21 words at random in one minute, 28 being the standard for success. He could not repeat 6 digits forward in any of three trials. He succeeded with three items and therefore was credited with 6 months.

He could not pass any of the 11 year items, and it was therefore assumed that he could not do any tasks even more difficult than this. A summary of his successes gives the following credits:

Basal age	6 years
7 years	10 months
8 years	8 months
9 years	4 months
10 years	6 months
11 years	0 month
Total	6 years, 28 months

His total mental age credit was therefore 8 years, 4 months. On the basis of a chronologic age of 11 years 0 months, he was given an I.Q. of 76. This classified him in the borderline group for general intelligence, and, since this test reveals particularly the kind of abilities necessary for school success, gave some understanding of his school difficulties. Inasmuch as he was not able to do any of the test items at his own age level, it was clear that his mental development was generally retarded so that he was below average in all test activities. This is the more usual picture in a case of low intellectual endowment: a steady slowing of growth, development, and learning ability. When low test scores are the result of some interference with mental function, some factor which keeps the child from efficiently using the powers he has (e.g., organic brain pathology, mental illness, or emotional or personality disorder), the score pattern is usually much more irregular, with some successes at or above the age level, and other types of task done outstandingly poorly.

Since he had worked hard at the above tasks for about 45 minutes, the test session was completed with the following brief, less formal procedures.

Goodenough Draw-A-Man Test (6). He was asked to draw a picture of a man. This he did in rather peculiar style, drawing a large elliptical head with nose protruding to one side in profile, but with eyes and mouth centered as though for a full face drawing, and the ear placed on the side opposite the nose. The body consisted of a rough rectangle, with pole-like arms and legs attached, and fingers were designated by 5 single lines of equal length. The drawing was scored according to the Goodenough norms, which give credits for specified characteristics. R. received credit, for example, for presence of head, legs, arms, trunk, eyes, nose, mouth, hair, fingers, ears, eyebrow, and pupil. He received additional credit for correct proportions of trunk, correct number of fingers, correct proportion of feet, arms, and legs in two dimensions, and firmness of drawing line. The total number of credits was equivalent to the average score of a child 7½ years old.

This test is useful in at least three respects. In the first place, it serves as another indication of mental level or intelligence. Frequently, however, factors other than purely intellectual considerations affect the score. It has been observed that the child who is poorly adjusted socially has a less mature concept of people, and that his drawing frequently scores below his general mental level on other tests. Lastly, there are certain qualitative aspects of the drawing which are important. Many times the concept is superior to its expression in the drawing, and disturbance of space or form perception is revealed in the child's drawing. Defects such as this are often characteristic of organic brain disease. A stick-figure or a snowman is often an evasion, usually employed only by rather clever patients. A tiny figure in a corner of a large sheet of paper is often drawn by a timid, anxious child. Another insecure child may draw in short sketchy strokes. The child who is overly concerned with his physical condition may draw a nude figure, whereas most children indicate clothing.

In the particular case under discussion it was the qualitative features of the drawing which deserved special attention. The "mixed profile" would be suggestive of impairment of space perception, if the same difficulty were exhibited in other tests requiring use of space and form relationships. Later tests proved this was not the case. Isolated bizarre features such as this are also found in the drawings of many patients with hysterical symptoms, thus raising the question of psychogenic components in the boy's reactions and in his attacks.

Picture Stories. He was next given a series of pictures of everyday situations clipped from magazine illustrations and advertisements. His task was to tell a short story about each one, explaining what it might be about. Although there are standardized tests which employ a similar technic, as for example the Murray Thematic Apperception Test, the present selection of pictures is one used by this examiner alone. The pictures deal with children and adults in familiar situations (playing, eating, working, etc.) and, functioning as a *projective technic*, permit the child to express the situations, attitudes, and feelings which are uppermost in his mind. It has been noted that there is wide variability in the type of response made by different children. Some children merely describe the picture, "The boy is sitting down." Others interpret the person's feelings, either explaining them or not, "The boy is mad"; some children add, "because he can't play with the others." Still other children invent a practical reason for the situation, "They're getting ready for school," etc. R.'s descriptions were very matter of fact, "The boy's running to school and he doesn't want to be late," or, "The boy's kissing his mother and she's happy." This kind of description indicates a ready, practical, but somewhat superficial reaction to social relations, and not much sensitivity to deeper emotional attitudes. In content, the only significant feature was a rather open expression of dislike for school.

At this point, the first test session was ended.

Cornell-Coxe Performance Ability Scale (3). On the second day he was given a performance test, measuring his general mental development and ability in *doing* things with objects rather than *saying* things, as in the more highly verbal Stanford-Binet examination. The performance scale is made up of a number of separate tasks or series of tasks, each of which receives credits. The total number of credits is then given a mental age equivalent according to tables which provide the corresponding mental age for each total score. This patient was given the Cornell-Coxe Performance Ability Scale. Similar information might have been obtained through the Arthur Point Scale, or the Pintner-Paterson Scale. The separate items described below are sometimes used individually as tests, but the scale or battery has greater validity as a measure of ability, since it is based on a broader sampling of behavior.

The following tasks were included:

Manikin and Profile. A manikin figure cut into 6 pieces must be reassembled, and a profile head with 3 face pieces and 4 ear pieces must be put together. Speed and accuracy are credited. R. put the man together correctly, but at the end of the 5 minute time limit had only the face pieces and 2 ear pieces of the profile in place.

Block Design Test. One-inch painted cubes with 4 solidly painted sides and 2 two-colored sides must be assembled to reproduce a design painted on a small card. Speed and accuracy are again credited. R. got the idea quickly on demonstration, and did the first 2 four-block designs easily. He was totally unable to get the next one, however, and was therefore not given the 9 and 16 block designs, since he seemed to have reached the limits of his ability.

Picture Arrangement. Series of cartoon-like pictures tell a story when arranged in the right order. They are presented in a mixed-up order, and the child must arrange them correctly. The items are of increasing complexity and have from 3 to 6 cards per series. R. was able to go through to the end of the series, making some errors, but with a good grasp of the tests. He received his highest point credit on this item, showing especially good ability to recognize the implications of pictured situations and analyze the proper sequence of events.

Digit–Symbol Test. Ten digits are presented at the top of the page, each accompanied by a simple symbol. Below this are the same digits alone in random order, and the child must fill in the symbol belonging to each, by referring to the key. He is credited for the number he marks correctly in a 2 minute period. A low score on this test is usually found with mental disturbance. R.'s score was intermediate between his highest and lowest.

Memory for Designs. Line drawings are presented on a small card for 10 seconds, after which the child must draw them. The first 3 cards have 1 design each, the fourth has 2. R. had the easiest one totally correct, confused the relationship between parts in the next but had all parts present, omitted features of the next two, and could not remember the last one at all. His total score was neither outstandingly good nor outstandingly poor.

Cube Construction. A model of a cube structure is presented with some faces painted and some unpainted. Nine small single cubes are also presented with just enough faces painted to complete the structure correctly. The task requires careful attention and analysis, as well as constructive talent and ability to visualize three-dimensional structures. Credits are given for accuracy and speed. R.'s score on these items was poor, with 3 to 6 blocks correctly placed in each. This, together with his poor score on block designs, emphasizes his intellectual limitations in logical analysis of complex situations.

Picture Completion. A sequence of 10 pictures represents the story of the same little boy in varied activities on the same day. Each picture has a small block cut out, and the child must select from a large group of blocks the one which will complete the picture most meaningfully. R.'s score was next to his highest, and confirmed his good ability to recognize the practical inferences of everyday situations.

Educational Achievement Tests. On the same day on which the performance tests were given, R. was also given part of his school achievement tests. It was estimated that he should be capable of work ranging from third to fifth grade, depending on the degree to which the material could be handled in a concrete manner.

This is computed in the following manner, to give a rough estimate of capacity level. A mental age of 6 years is required for successful first grade work, 7 years for second grade work, and so on. Therefore, mental age less 5 gives a rapid estimate of educational capacity.

R. had a mental age of 8 years 4 months on the Stanford-Binet scale and 10 years 3 months on the Cornell-Coxe scale. His expected level is third to fifth grade, probably centering nearer the lower end of this range since verbal abstract abilities are more important in determining school success.

A number of school achievement tests are in wide use, all based on the same principle of standardizing the average number of correct responses among school children at various grade levels. R. was given the Progressive Achievement Test (12). His test scores were as follows:

Reading vocabulary, 4.8 grade.
Reading comprehension, 4.2 grade; total reading, 4.4 grade.
Arithmetic reasoning, 4.1 grade.
Arithmetic fundamentals, 3.4 grade; total arithmetic, 3.8 grade.
Language, 4.3 grade.

Total score, 4.1, grade; age equivalent 9 years, 6 months.

These tests require several hours to complete, and were therefore administered in two sessions. He was restless, and fidgeted and squirmed while he worked at them, making no effort to conceal his dislike for school work. While he was working on the arithmetic, he had two brief seizures in rapid succession. This was of some interest, since the onset of seizures was first noted in conjunction with arithmetic homework and since hysteroid features were noted in some of his test reactions. He became noticeably more relaxed as soon as the activity was shifted to something which he enjoyed.

The grade rating on these tests indicated that he was doing as well in school as could be expected, and that his failures were due to limited capacity rather than to poor motivation or lack of desire to succeed. Arithmetic was harder for him than other subjects, but the discrepancy was not so great as to suggest specific disability requiring remedial measures.

Stenquist Mechanical Assembly Test. At the third and last test session he needed about 45 minutes to complete school tests. Following this he was given a mechanical aptitude test, since he had done relatively well on concrete manipulative tasks. The Stenquist Assembly Test is made up of a series of 10 objects of increasing complexity which have been disassembled and which the child must put together so that they will "work" within half an hour. The objects are: cupboard catch, wooden spring clothespin, spring paper clip, link chain, bicycle bell, rubber hose shutoff, wire bottle stopper, doorbell push button, door lock, and mousetrap. He was able to assemble 3 of these completely correctly, and had 2 more partially correct. His score was a little above that for average 12 year olds, indicating special talent in this kind of mechanical task, since he scored at a higher level than on any other type of item.

Rorschach Test. This was the last test procedure used. It is usually well to reserve this procedure until late in the course of examination, since the child is more at ease and less easily disturbed by demands which he cannot quite understand. The first blot R. interpreted as a bat in flight, which is the most popular interpretation. The second card was described as a crab, which is not a popular response and uses the form elements only in a vague way. Cards III and IV he rejected as meaningless. Card V was described as a butterfly, using a simple popular interpretation of the total form. Card VI was rejected. In the next he saw two dogs in the act of putting their mouths against an object. Card VIII was rejected. Card IX was described as a man's head, and in the last card he described two green worms. The significant features of this record were noted as follows: (*1*) Low general productivity, with rejection being a typically neurotic reaction, indicating that he is restricted by emotional factors. Normal, well-adjusted subjects rarely reject more than one card. (*2*) The general quality of interpretations was either popular or poorer, with less accurate use of form indicating less than average intelligence. More intelligent people have a few interpretations occurring only a few times in every hundred records, which nevertheless conform closely to the form of the inkblot. (*3*) The most meaningful aspects of the record were its areas of omission—absence of originality, lack of variability of concept, lack of many responses, lack of use of shading or color or free introduction of movement, lack of accurate use of detail. On the basis of these positive and negative qualities, the following interpretation was made: "This is a somewhat dull child who does not function very creatively even in the absence of emotional stress, and who is totally incapacitated by anxiety in strange or new situations."

This ended the psychologic examination. On the basis of the findings, the following recommendations were made and are being carried out. He is being moved to a school which has more varied facilities and a more flexible program. He will be taught academic subjects in a group of other children equally retarded, so that he will not be expected to complete a full grade's work each year, and will be taught in as concrete terms as possible. He will also be able to compete with his classmates on an equal footing. Only part of his school day will be spent on academic work, with which he will always have difficulty; the rest of his time will be devoted to manual training and shop activities, at which he can do average or better work. This will prepare him for a vocational high school course in which he can receive specific trade training. It is to be hoped that he will overcome his anxiety in new situations when he has learned by experience that he can succeed, and when he has been praised deservedly for acceptable accomplishments. It is likely that he will respond to the unspoken attitudes of acceptance on the part of other people because he has shown alertness to the implications of social situations, and has exhibited the potentiality for good personality adjustment. Freedom from stresses and pressures should also remove any emotional or hysterical factors which tend to aggravate the occurrence of attacks. There was no evidence at any point of significant interference with mental function. There was no reason to believe that he was doing more poorly than expected within the limitations of his native endowment. Other clinical examinations did not suggest any pathology with which mental impairment might be expected. Neurologic examination was negative. Electroencephalographic examinations revealed "an abnormal pattern highly characteristic of epilepsy" but there was no evidence of a focal lesion.

CASE II

O., a 10 month old girl, was born prematurely at 7 months, and kept in an incubator for 3 months. After an additional month of convalescent care, she was placed in a foster home by a social agency. At the end of 6 months, the foster parents expressed a desire to adopt her; psychologic examination was requested by the agency to determine whether she was suitable for adoption with regard to her mental development. There were no complaints or problem behavior.

The child was a healthy looking baby. For most of the examination she sat on the foster mother's lap and amused herself by playing with colored blocks and toys on the desk. She smiled responsively when toys were extended to her. Part of the time she crawled on the floor and made primitive movements toward raising herself to a standing position while grasping a support.

She was first given the Kuhlmann Infant Tests (9). The test presents a situation which is most likely to elicit the desired behavior. In some instances, however, the child may fail to respond in the expected manner, even though he is capable of the act. For this reason, credit is given also for items the child failed, if the parents present satisfactory evidence that he can perform the act at any time.

O. was credited with all 6 month items. She sat erect and balanced her head when her back was supported, and for much longer periods than the required 5 to 10 seconds when she was not supported. She turned her head promptly toward the source of sound of a small bell. She grasped a one-inch cube with the thumb forcefully opposed to the fingers. She reached toward an object held before her.

At the 12 month level, she sat unsupported for an indefinite period. She imitated several movements, such as nodding her head, and waving her hand. She showed preference for a rubber ball among several toys, and selected it when it was placed in several different positions among the other toys. She failed to combine syllables in speech and the mother could report no occasion on which she had said ba-ba, ma-ma, etc. She also made no effort to mark on a paper when a pencil was placed in her hand and the examiner both demonstrated marks and guided her hand in making some. Each of these items was credited with $1^1/_5$ months, giving her a total credit of $3^3/_5$ months at this level.

She passed no items at the 18 month level. The tasks were as follows: drinking several swallows in succession from a glass; feeding herself with fork or spoon; speaking any word "more or less distinctly"; spitting out distasteful solid food; showing recognition of familiar objects in pictures. Her total rating was, therefore, $9^3/_5$ months, or approximately 10 months.

On the Gesell schedules, she was compared with standards for motor development, language development, and personal–social development. In motor development, she was nearest the 40 week standard with relation to sitting, attempting to stand, and grasping. Language development was similar to 32 and 36 week standards. There was some vocalization and enunciation of syllables, and alert response to the sound of her own name, but no imitative repetition of syllables. Personal–social development was rated at 36 and 40 weeks, with holding her own bottle, feeding herself a cracker, and waving bye-bye.

A third rating device was used to confirm the results of these tests, although many of the individual items are similar. In the Vineland Social Maturity Scale, the parent or other person thoroughly conversant with essential details describes the child's habitual behavior, and credits are given for individual items (4). The total score is converted into a "social age equivalent" by reference to a table. O. was given a social age of 0.85 year, which is approximately 10 months. The items are of the same character as those described above: vocalization, sitting unsupported, attaining a standing position, drinking from a glass, grasping objects firmly, recognition of familiar people, etc.

On the basis of these procedures, the following summary was made: "This child is of at least average rate of development. It is difficult to evaluate the effect of her first three months spent in an incubator, since the length of time she has been exposed to a normal varied environment and its learning experiences has been briefer than for most children. It may be that she has already compensated for this initial deprivation, or it may be that her learning and general maturation will still be accelerated. Efforts at vocalization and language do not seem to be as advanced as her motor development, but this has often been noted in babies who are developing rapidly in their motor powers, and enlarging their domain by 'going after' things rather than by 'asking for' them. At the present time it is certain that she has developed normally and without defect to this point, and future careful observation might prove her to be even a little quicker than the average. She would be a good adoptive risk in her present home in which she has made an excellent adjustment."

SUMMARY

In summary, we note that the psychologic examination has as its main purpose an evaluation of the child's mental abilities and personality characteristics. Its goal should be the practical application of the obtained knowledge to planning for the most effective use of the child's abilities and his most congenial adaptation to the environment. In many instances it is customary to make such a study only if there is clear evidence of maladjustment. There is growing recognition of the value of using the psychologic examination as a precautionary measure, to ensure suitable planning for the child, and to avoid possible maladjustment.

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CHAPTER XIV

Tests of Hearing and Sight

HEARING TESTS

Hearing can be tested quantitatively (tests of acuity) and qualitatively (tests which differentiate between the various forms of impaired hearing on the basis of etiology).

In the acuity tests one determines the minimal audibility for the human voice, for musical tones, or for sounds such as a whistle or monochord. Informal methods are those employing the whisper, the spoken voice, and the noises of watch, whistle, bell, etc. These procedures do not measure acuity with exactitude; they do, however, provide a rough estimate, and screen out individuals with doubtful acuity of hearing.

The tests using musical tones produced by tuning forks are more accurate, the most precise measurement being achieved by the audiometer, an electrically operated instrument. Tones of any desired number of vibrations, of any strength, and of any amplitude can be produced with this instrument, and the minimal audibility for every tone can be measured. The results of such a test are plotted on a chart.

Qualitative tests help to determine the nature and site of the lesion responsible for the deafness. For instance, Rinne's test, consisting of a comparative determination of air and bone conduction, demonstrates whether disease is confined to the middle ear or whether the inner ear and auditory nervous pathways are also involved.

Vestibular function tests, which examine the response of the labyrinth to rotary, caloric, or galvanic stimulation, supplement the results of hearing tests. The reactions of the labyrinth, disclosing whether the vestibule and the semicircular canals have been affected by the disease, permit important conclusions as to diagnosis and etiology.

PEDIATRIC CONSIDERATIONS

Spontaneous reactions to sounds in the full term infant do not occur before the fourth day of life; they can be first verified during the first and second weeks of life. However, the existence of auditory sensation can be demonstrated even on the first day of life by an induced reaction to sound—the auriculopalpebral reflex. Spontaneous reactions consist of reflex-like motor movements of various parts of the body. During the second to fourth months, the child responds to sounds by turning the head and looking toward the source of the sounds.

Auditory function tests may be carried out during earliest infancy, but their success depends upon the selection of proper methods. Since the child's potential functions mature with advancing age, the choice of tests is dictated by the patient's mental faculties and by the ability to memorize and discriminate between sounds of different pitch, and to understand the human voice. The younger the child, the less useful are tests designed for adults, and the smaller is the number of tests that may be used.

Almost the only tests needed for infants and children under 2 years are those that will show whether complete or marked deafness is present. The sole dependable method during infancy is the elicitation of the auriculopalbral reflex. A less reliable criterion of deafness is the infant's reaction to the sounds of whistle or bell: the majority of normal newborn infants cease crying and become quiet when a small bell is rung (1); one-fourth show no observable reaction to the sound of a whistle.

In children over 1 and under 3 years of age with a positive auriculopalpebral reflex, the informal, nonquantitative test methods can help to detect impaired hearing (reactions to various sounds and noises). Between the third and sixth years, audibility for the human voice, whispered or spoken, can be determined quantitatively. By the end of the sixth year, the child is generally able to respond reliably to tests performed with tuning forks, but examination with fork C-4 is rarely possible in children younger than 9 to 10 years. Electric audiometer tests give accurate results in most children over

6 years old. Tests which may be used in infants and children, and the ages at which they may be used, are given in Table 64.

Of the qualitative tests, Schwabach's test may yield reliable results in children between the ages of 6 and 8, whereas Weber's and Rinne's tests are not feasible in children under 10 years of age.

Labyrinthine function can be tested successfully in any child, without regard to chronologic or mental age. Vestibular reactions are elicited as promptly in the child as in the adult.

TABLE 64
Earliest Ages (in Years) at Which Various Hearing Tests May Yield Reliable Results

Test	Age
Auriculopalpebral reflex	Neonatal period
Quantitative tests	
Speech (whisper)	3–5 years
Tuning fork	6–10 years
Audiometer	6 years
Qualitative tests	
Schwabach test	6–8 years
Weber test	10 years
Rinne test	10 years
Calorific stimulation of labyrinth	Neonatal period

Measurement of a child's auditory function is complicated by his relative immaturity. Possibilities of erroneous interpretation are not primarily of a technical nature, but the result of missing, doubtful, or unsolicited responses by the child to properly applied tests. Failure and success depend largely on the selection of methods that are adequate for the child's potential reactive abilities. An understanding of the physiologic limitations and a knowledge of the few simple procedures which are applicable to infants and children are the only qualifications needed for performing these tests. Obviously, the examination must be limited to tests that detect rather than measure impaired hearing. When a physician suspects that an infant is deaf-mute, he faces the alternative of concealing his belief or of disclosing it to the child's parents. If he evades the responsibility of identifying a condition which later becomes obvious, he risks his reputation. The pediatrician should therefore make use of the available, simple test methods for early detection of impaired hearing. The surprising disinclination of otolaryngologists to perform hearing tests in infants and young children makes such a course almost mandatory. For complete studies, of course, the skill of a specialist is indispensable. In assembling the data on the test methods described on the following pages, use has been made of Alexander's treatise on ear disease in children (2).

AURICULOPALPEBRAL REFLEX

PROCEDURE

The child is held by the mother or nurse, preferably on her lap. Young infants may be examined lying in their crib. The child's attention is engaged by holding a toy, a watch, or some other object in front of him. While so doing, the examiner must scrupulously avoid making any noise. When the child is looking straight ahead and a little upward at the object, a person standing behind the child produces a loud noise or tone. At the moment of the auditory stimulation, the child will blink his eyes.

It is essential that neither the source of sound nor any manipulation connected with its production becomes visible to the child, since this may produce an optical instead of an auditory reflex. Tactile sensations must also be carefully avoided, particularly if clapping of hands is used as a stimulant.

According to Alexander (2a), the tuning fork is the object of choice for producing sound. The vibrating fork is brought close to one auditory meatus in such a way that the tone can be perceived by the child, but optical and tactile reactions are prevented. This is the most accurate form of testing.

If the test is carefully performed, one may rely on the reactions to the first attempt. If the test has to be repeated, it is best to wait until the following day, since repetition at short intervals may cause a failure of reflex, even in the presence of auditory function.

Another procedure tests each ear separately. A wisp of cotton soaked in paraffin oil is placed in the meatus of one ear, while the other ear is being tested. However, experience has shown that the presence of cotton in the ear very often irritates infants and children to such a degree as to interfere with the test. Barany's noise apparatus has also been suggested; precautions against eliciting other than auditory reflexes are particularly indicated.

Absence of the auriculopalpebral reflex is evidence of deafness. A positive reflex proves the presence of auditory sensation.

CLINICAL APPLICATION

Elicitation of the reflex is the most reliable test of hearing in early childhood, even when loss of hearing is associated with idiocy. There are no other means of proving as surely the presence or absence of some degree of useful hearing. However, difficulties and errors are inherent in all tests applied to infants and young children, and the physician should evaluate the test results most carefully before giving a definite opinion.

The pediatrician will find the test most useful in such situations as:

(*1*) When asked to pass on a child's ability to hear because the parents believe the child does not respond to sudden noises in the usual way.

(*2*) When parents, because of a history of familial deafness, desire certainty as to their child's hearing.

(*3*) When loss or impairment of hearing due to congenital atresia of one or both auditory meatuses must be established.

(*4*) When a child is being examined because of failure to develop speech. In such cases, deaf-mutism, functional mutism, aphasia, or idiocy may be the underlying cause. A negative auriculopalpebral reflex rules out all but the first-mentioned, whereas a positive reflex eliminates deafness as the causative factor.

The outcome of vestibular function tests (page 436) shows whether deafness is congenital or acquired. Simultaneous absence of the auriculopalpebral reflex and of caloric nystagmus is pathognomonic of acquired deafness, while a prompt labyrinthine reaction in the absence of the auriculopalpebral reflex is almost always evidence of congenital deafness. Not infrequently, however, congenital deafness is not complete, and a remnant of auditory function causes a positive reflex (2b).

REACTION TO HUMAN VOICE (WHISPER OR SPEECH)

With an identical technic, whispering or the ordinary speaking voice is used as a stimulus. The test described here is the whisper test, the more frequently used of the two tests.

PROCEDURE

The examiner stands behind or at the side of the child, in such a way that his lips cannot be seen or read. Each ear is tested separately, a wad of cotton soaked in paraffin oil being placed in the meatus of the ear not being tested. The examiner standing on successive lines marked off every foot up to 25 feet, determines the furthest distance at which whispered numbers, chosen at random, can be heard and repeated by the patient. Results are recorded in terms of the fractional part of normal acuity; for example, 8/20 represents hearing at 8 feet which should be heard at 20 feet.

There is a wide divergence in opinion as to what should be considered normal acuity, as measured by this test. The normal limit of hearing a whisper in a quiet room, as given in different textbooks, varies between 5 and 40 meters. The majority of the otologists questioned by the writer considered 6 meters (20 feet) as the average distance at which the whispered voice should be heard by subjects with normal acuity. In the U. S. Navy Air Corps (3), the whisper test is performed at 15 feet distance. On the other hand, by U. S. Army (4) and Canadian Army (5) standards, hearing of a low conversational voice at 6 meters is considered normal.

Children who do not hear accentuated whispering at a distance of 20 feet are probably hard of hearing, and require exact function tests.

RINNE'S TEST

Each ear is tested separately, the auditory meatus on the opposite side being blocked by an oil-soaked wisp of cotton or by the nurse's finger pressing down the tragus. The test should not be started until the child has gained confidence in the examiner and can be expected to cooperate.

PROCEDURE

A vibrating tuning fork, usually of 436 d.v., is held to the meatus at a distance of about 2 centimeters, and the child is asked to state the moment he ceases to hear the tone. The fork is then instantly transferred to the mastoid, the handle of the fork being applied to the skin overlying the mastoid bone, and the patient is asked whether he hears the tone. If it is not audible, the test is reversed, the vibrating fork being first applied to the mastoid; the moment the child indicates that the sound has faded away, the fork is brought close to the meatus to find out whether the tone is still audible there.

The results of the test are positive (Rinne positive or R+) when the sound is heard for a while at the meatus (through air conduction) after it is no longer audible on the mastoid (bone conduction). The results are negative (Rinne negative or R—) when the vibrating fork is heard on the mastoid after it has ceased to be audible at the meatus.

The results may also be reported more completely as the difference in seconds between air and bone conduction. For example, if audibility by the air path (meatus) on the left side with fork C-2 is 15 seconds longer than by the bone path (mastoid), the result is noted as C^2L+15. Correspondingly, C^4R-10 designates a negative result with the right ear, the bone conduction time being increased by 10 seconds over the air conduction, when tested with fork C-4.

INTERPRETATION

In persons with normal hearing, air conduction of sound outlasts bone conduction by an average of 15 seconds, so that the test is positive.

In those who are hard of hearing, a positive response to Rinne's test indicates that the inner ear is affected, and that bone conduction is reduced (perceptive deafness).

A negative result is due to impaired air conduction. It is found typically as a sequela of acquired or congenital disease of the middle ear (transmission deafness), and may also be present when loss of hearing is caused by obstruction of the auditory meatus (congenital atresia).

When both air and bone conduction are reduced to an equal degree, Rinne's test remains positive. When both the middle and internal ear are affected by disease, but in different degrees, the test discloses which part has suffered most.

WEBER'S TEST

A vibrating tuning fork, preferably of 108 d.v., is applied to the child's forehead at the midline and the patient is asked to localize the sound he hears. Children with unimpaired hearing will refer the sound to the midline, to both ears equally, or will hear it diffusely all over the head. In unilateral deafness due to disease of the middle ear, the sound is referred to the diseased ear; in labyrinthine deafness or impairment of the auditory nervous pathways of one side, the tone is heard in the normal ear. In complete bilateral deafness, no sound is heard at all. The test demonstrates differences in air or bone conduction between the two ears.

SCHWABACH'S TEST

The duration of the patient's bone conduction, as compared with the normal standard is determined, the examiner's hearing power serving as normal. Each ear is tested separately, the opposite side being blocked as described for Rinne's test. If done in the classic manner, a vibrating tuning fork of 108 d.v. is placed on the median line of the vertex so that it rests directly upon the scalp, the hair being separated. Pressure upon the fork must be avoided. When the patient states that he no longer hears the sound—actual tones, not vibrations—the fork is removed for a second because of the so-called "fatigue symptom," and then is replaced. When tone is no longer perceived, the fork is set upon the examiner's head, provided his bone conduction is known to be normal. The length of time, in seconds, which the examiner hears the sound after it is not audible to the child gives a measure of the hearing loss. The fraction 40/32, for example, represents a "prolonged" response to the Schwabach test, the numerator referring to the audibility for the patient, the denominator to that for the examiner. Correspondingly, 32/32 and 18/32 would designate normal and reduced responses to the test.

INTERPRETATION

Lengthened bone conduction usually means impairment of the conduction apparatus. It is a characteristic sign of disease of the middle ear, particularly otosclerosis and adhesive processes. Shortened bone conduction indicates some form of inner ear or auditory nerve lesion, such as are due to suppurative or degenerative processes, lues, tumors, and trauma.

Vestibular Function Tests

Labyrinthine function is tested by applying various stimulants and observing the response to them. Stimulation is achieved by means of rotatory, caloric, or galvanic excitation of the vestibular apparatus. The only form appropriate for pediatric use is the caloric stimulation; all other tests of labyrinthine function lie within the otologist's province.

CALORIC STIMULATION

The test cannot be performed if there are punctured dry ear drums. Each ear is examined separately.

PROCEDURE

The child's head is either held upright or bent backward at an angle of 30 degrees. One ear at a time is syringed with cold water of 27 C. (80 F.) for about a minute. In many institutions, 5 cc. of ice water are used, or 5 cc. of water at 27 C., instilled into the external auditory meatus with a drop pipet (6). For children, irrigation is to be preferred. This is done by allowing the water to run from an elevated glass container through a long rubber tube into the conic glass tube that is inserted into the meatus, thus safely avoiding syringing under unduly high pressure. Water warmer than body temperature, about 45 C. (113 F.) may be used instead of the cold water.

INTERPRETATION

Reactions to the caloric stimulant should become manifest within 15 to 30 seconds. They consist of horizontal nystagmus when looking straight ahead, vertigo, and past-pointing.

In infants and young children, the appearance or nonappearance of nystagmus is the only reliable criterion of the labyrinthine response. Cold and hot water produce deviations in opposite directions. The average duration of the nystagmus is 1 to 2 minutes. Failure of caloric stimulation to cause nystagmus indicates a lesion of the labyrinth or of the nervous pathways. For an accurate, localized diagnosis, a complete analysis of the postrotatory reactions is needed.

The diagnostic significance of the caloric test in differentiating congenital and acquired deafness has already been discussed (page 433).

Testing for Deafness at Various Age Periods

In pediatric practice it is primarily the early detection of deafness that calls for functional examination of the ear. A practical example will best illustrate how the physician may utilize and evaluate the limited number of tests at his disposal.

M. is 1 year old, and has been exhibiting signs of behavior and development strongly suggesting the presence of congenital deafness. There is no history of ear disease, and the parents are unaware of the situation. The pediatrician confirms his clinical impression by a series of tests, in the following sequence.

(*1*) *Auriculopalpebral Reflex.* This is found to be consistently negative, regardless of the ear examined and the source of sound utilized. Conclusion: the child has no perception of sound.

(*2*) *Caloric Stimulation.* Horizontal nystagmus appears promptly upon irrigation of either ear with cold water, demonstrating an intact vestibular irritability. From the combined results of the two tests, a diagnosis of congenital deafness, caused by lesions of the inner ear which are restricted to the sacculocochlear apparatus, can be made almost with certainty.

The parents are told as tactfully as possible. For the following 2 years nothing can be done except to repeat the two tests at intervals. By the end of the third year, the child's failure to develop speech has become obvious (deaf-mutism). The time has now come to make use of all available school facilities in order to prepare the child for re-education and to assure continuous advance in mental development. At the age of 5 years, renewed attempts to elicit the auriculopalpebral reflex are successful, and it is repeatedly positive when the left ear is stimulated, proof that some isles of useful hearing remain on the left side.

As the child grows older, several attempts to test his hearing quantitatively and qualitatively yield negative results. Finally, at the age of 6½ years, Schwabach's test is carried out with a fair degree of accuracy, and auditory function is shown to be $^5/_{32}$. It is followed up with Weber's test, in which the child indicates slight sound perception on the right side.

The results obtained thus far substantiate the original, tentative diagnosis of congenital inner ear deafness (perceptive deafness). The presence of a small degree of useful hearing in the left ear leaves hope for some speech development.

VISION TESTS

Eye tests serve different purposes in the various age groups. Its primary object in infants is early recognition of congenital anomalies which interfere with perception of light and cause severe visual defects or blindness. The most important test for such gross abnormalities in the neonatal period is elicitation of the eyelid reflex. Supporting information may be obtained by observing fixation movements which normally become apparent during the second or third week of life. Gross visual defects that have not been recognized during the first weeks of life invariably become manifest, even to the layman, sooner or later during infancy. Since nobody observes a child with greater care and apprehension than the mother, the first intimation that something may be wrong with a child's vision usually comes from the mother. It is the physician's task to dispel or confirm her dread. The crucial test is the eliciting of the eyelid reflex.

Vision testing, using tests measuring distance vision, can be carried out in children 3 years of age and over. The pediatrician uses these tests merely to discover the individuals requiring oph-

TABLE 65
Data on Visual Tests in Infants and Children

Age	Stimulus	Response	Assumption as to function
First 10 min. through newborn period	Flashlight	Eyelid reflex	Retina functioning
2–3 wks.	Flashlight	Eyelid reflex and convergence reflex	Macula starts functioning
5–6 wks.	Flashlight or massive objects	Eyes follow movements of object for few seconds	Developing function of macula and of muscle coordination
6–8 wks.	Smaller objects	Eyes follow movements of object for few seconds	Developing function of macula and of muscle coordination
3–4 mos.	1 inch black cube against white surface at 2 ft.	Eyes follow movements of object for ½ min.	Visual acuity about 6/728
6 mos.	⅓ in. black cube against white surface at 2 ft.	Movements of object followed through full angle of eye movements	Visual acuity about 6/288
9 mos.–2 yrs.	Black cubes of gradually decreasing size against white surface	Movements of object followed through full angle of eye movements; hands and body in prolonged attention	Development and coordination of all eye functions; visual acuity about 6/72
3–5 yrs.	Symbol E chart	Indicating position of letter	Visual acuity 6/12–6/9
6 yrs. and over	Snellen charts or modifications	Reading of letters, etc.	See Table 66

According to Evans (8).

thalmologic attention (7). For children who are able to read, the Snellen charts are the most accurate and simplest devices. Children between the ages of 3 and 6 may be tested by using charts showing pictures of drawings of well-known objects. The Symbol E charts are also widely used for testing this preschool group.

There is a wide gap in vision testing between early infancy and the age of 3. The chart methods are not yet applicable, and even ophthalmologists are often at a loss for ways of ascertaining suspected impairment of vision in these children. A few simple devices are available, which, according to Evans (8), "do not measure visual acuity but rather a condition of responses to brightness, contrast and movement." The pediatrician will find these test methods extremely useful.

As a rule, marked impairment of vision affects the child's mental function, and can be recognized by abnormal behavior, by lack of normal reactions to the surroundings, and often by the absence of normal mental growth. Psychologic factors may also contribute to the outcome of visual tests in young school children, especially those who do not yet read easily.

Table 65 lists the tests of visual function which may be considered most useful, dependable, and simple enough to be employed in pediatric practice.

EYELID REFLEX

A strong light is directed straight into the infant's eyes. If the subject perceives light at all, i.e., if he is able to differentiate between light and darkness, flashing of a light before his eyes will elicit contractions of the orbicularis oculi muscle (eyelid reflex). This response can be obtained even during the first hours of life, regardless of whether the infant is awake or asleep. Absence of the reflex indicates blindness. In making the test, every precaution must be taken to cause no acoustic or tactile irritation of the child.

In infants who are several months old, the eyelid reflex may also be elicited by rapidly thrusting an object close to the child's eye.

RESPONSE TO CONTRAST, BRIGHTNESS, AND MOVEMENT

These tests demonstrate vision in children between 1 and 3 years of age. They require greater skill and patience on the part of the examiner than vision tests in all other age groups. As mentioned before, the methods do not measure visual acuity; they are devised to examine the child's behavioral reaction to various test objects with which he is confronted. Any reaction, when it occurs, can be considered as proof that the object has been visualized, i.e., that its shape has been perceived and formed into a distinct mental image. Obviously, the actual reaction is also conditioned by the child's mental development.

Various devices have been recommended as test objects, all of them being constructed on the principle of small objects being moved about against contrasting backgrounds. For instance, a white ball mounted on a black stem is moved on a dark ground; a black cube is moved against a white surface; iron fillings or steel ball bearings are caused to move about on a white surface (framed, 6 inch square tray with replaceable opaque plastic bottom) by means of a bar magnet manipulated on the underside of the tray (8). This modification has the advantage that the examiner's hand can be kept out of the field of the subject's vision.

PROCEDURE

The infant or child, held on the mother's lap or seated on a table or in a high chair, is faced with the test object at an appropriate distance. Care should be taken that illumination is satisfactory and that the object casts no shadow on the contrast background. When the child's attention has been attracted by the test object, one of the eyes is screened off by the mother, and the test starts. If the child follows the moving object with interest, grasping or pointing to it just as he did when looking with both eyes, it may be concluded that the object has been visualized. If the child does not succeed in finding the object with his one exposed eye, he will endeavor to remove the mother's screening hand from the other eye in order to see the moving object again. Evans (8) states:

> "It is this reaction on the part of the infant which insures the dependability of the test even more than the child's unsuccessful efforts to find or follow the movements of the object."

One begins the test with small sized objects, which are not expected to attract the child's attention easily. Then one continues with objects gradually increasing in size, until the child detects and follows them with his eyes. In this way, a kind of quantitative measurement is attained. Table 65 gives the average normal reactions according to age.

Distance Vision Tests (Central Acuity)

TEST WITH SYMBOL E CHARTS

These charts, also called illiterate E charts, show lines of E letters of Snellen scale, the E letters appearing in different positions —up, down, sideward, etc. The various lines correspond to the lines of the Snellen chart in size, interval, and designation. Visual acuity is expressed as a fraction (see Snellen test, below).

The E charts are the best available device for testing preschool children between the ages of 3 and 6, but they may also be used for older children.

The instructions for performing the E chart tests are taken from a pamphlet issued by the New York State Commission for the Blind (9).

PROCEDURE

Four cards of soft, light gray cardboard, 9 by 11½ inches, are used. One of the four cards has no holes whatever, and is used to cover the part of the chart commonly not in use; the other cards have various sized holes to show single letters on the different lines of the chart. Using a separate card on which is displayed the letter E drawn to the exact scale of the 200-line on the Symbol E chart, the child is taught the various positions which the letter E can assume —up, down, right, left—until he becomes familiar with them at close range. This should be done as though it were a game. The same figures must then be recognized through the round hole. Time and patience are necessary in these initial steps, if the game is to be played with any degree of success.

After the child has become familiar with the procedure, he is placed at the 20 foot mark in a straight line with the hanging chart. The right eye is tested first, then the left eye, then both eyes together. When the child being tested is small, an assistant holds a 3 by 5 inch card over one eye, with the card placed obliquely across the nose in such a way that all vision of the covered eye is obscured. Both eyes remain open during the test.

As the examiner shows the symbols through the cover card apertures, the child indicates with his hands the way the E points.

Reading one vertical and one horizontal symbol on a line with each eye and with both eyes together is considered satisfactory evidence of normal vision. Testing of children suspected of low vision begins at the top of the chart; with other children it may begin with the 50 foot line and proceed to include the 20 foot line. There is no need to test beyond the 20 foot line.

TEST WITH SNELLEN CHARTS

The charts are used for children able and willing to read, and are routinely employed in screening tests of school children. The Snellen charts, or their modifications, show letters or figures of varying size, arranged in lines, each row composed of letters or figures of identical size. The actual size of the letters and the intervals have been standardized by international agreement. The test charts are hung at a distance of 6 meters (20 feet) from the patient and approximately on a level with the child's eyes. Shorter distances are indicated only for those who cannot see at the 6 meter line. Artificial illumination of the chart is advisable, preferably by blue bulbs. A number of commercial devices are available for standard illumination. Light in the examination room should not be less than one-fifth of the chart illumination.

PROCEDURE

Each eye is tested separately, the other eye being screened off in the manner described above. Aperture cards need not be used. Testing usually is started at the 30 meter (100 foot) line and proceeds to the 6 meter (20 foot) line. A row is considered satisfactorily read when there is no more than one error. Children wearing glasses are first tested with and then without glasses. Results are recorded for each eye separately, by means of the fraction next to each line on the chart. Thus in the fraction 20/32, the numerator gives the distance at which the letters are actually seen by the patient, the denominator the distance at which this line can be read by the normal eye. If the type of this particular line is the smallest one successfully read by the patient, it means that he is able to recognize this size at a distance of 20 feet, whereas a normal eye can read it at 32 feet. Some charts give, in addition, a percentage rating of visual efficiency.

INTERPRETATION

While the technic of testing children with the Snellen type of chart is essentially the same as in adults, the results are evaluated somewhat differently. A high percentage of healthy children show visual acuity which is below the normal adult standards. This is true even when allowance is made for psychologic factors which may affect the test results. As may be seen from Table 66, the percentage distribution of 20/20 vision increases with age. At 14 years, only 75 per cent of healthy children have the normal visual acuity of an adult. From then to about the age of 25 years there is probably a gradual increase of visual acuity, after which a very gradual decline begins which is not attributable to disease.

TABLE 66
Visual Acuity of Healthy Children at Different Ages

Age, years	Per cent with 20/20 vision	Age, years	Per cent with 20/20 vision
3–4	16	8–9	56
4–5	23	10–11	61
5–6	38	12–13	70
6	58	14 and over	75
6–7	52		

From Berens (10).

Children should be referred for opthalmologic attention if the visual acuity is below 6/9 (in meters), or 20/30 (in feet).

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CHAPTER XV

Endocrine Function Tests

THYROID GLAND

Laboratory methods have now been developed which are more satisfactory tests of thyroid activity in children than the determination of the basal metabolic rate. The changes in body chemistry which these tests reveal, and the abnormal responses to tolerance tests are of great value in the diagnosis of thyroid dysfunction. Hypothyroidism, particularly, should not be definitely diagnosed in children except when one of these chemical tests supports the diagnosis.

Table 67 gives a list of laboratory methods for measuring thyroid activity in children. All but the thyroid sensitivity test, which is discussed below, have already been described in preceding chapters. The following brief summary concerning choice of tests may aid the pediatrician.

Determination of the basal metabolic rate is not a satisfactory test method for young children. The difficulties of attaining basal conditions and of selecting the proper standards (pages 65–76) inevitably leave one with the feeling that the results of such breathing tests are "of questionable value in the diagnosis of hypothyroidism during childhood" (1). Certainly, it is no coincidence that the search for, and the development of, more reliable test methods were initiated in the very institutions in which basal metabolism tests have been practiced with traditional perfection. An abnormally high serum cholesterol level is commonly regarded as one of the biochemical manifestations highly significant of hypothyroidism (1). However, a normal serum cholesterol level in a child who has not received thyroid medication does not rule out the possibility of hypothyroidism (2). It is doubtful whether the total blood lipids are a better criterion of thyroid dysfunction, as has been suggested by Radwin *et al.* (3).

The response of the serum cholesterol to thyroxin administration provides even more accurate information with regard to thyroid function. Known as the thyroxin test, this method deserves wide clinical application.

Abnormally low phosphatase activity in serum is a very reliable index of thyroid insufficiency in infants and children. But the value of phosphatase determination as a test for thyroid hyperactivity is open to question.

TABLE 67
Biochemical Data Suggestive of Abnormal Thyroid Activity

Constituent or reaction measured	Characteristic changes (above or below normal) in: Hypothyroidism	Characteristic changes (above or below normal) in: Hyperthyroidism	Test method outlined on page
Basal metabolic rate........	Decreased	Increased	65
Serum cholesterol...........	Increased	Decreased	137
Alkaline serum phosphatase..	Decreased	Increased	211
Protein-bound plasma iodine.	Decreased	Increased	228
Plasma volume.............	—	Increased	233
Plasma Vitamin A (carotene).	Increased	—	251
Urinary creatine............	Decreased	Increased	170
Urinary 17-ketosteroids.....	Decreased	—	464
Glucose tolerance...........	Increased	Decreased	103
Iodine tolerance............	—	Increased	227
Creatine tolerance..........	—	Decreased	176
Galactose tolerance.........	—	Decreased	115
Thyroxin sensitivity........	Increased	—	449

The blood content of protein-bound iodine is very significant, clinically, particularly in the diagnosis of borderline cases. A wider use of the method in children is limited by the technical difficulties involved.

Galactose tolerance is characteristically altered in hyperthyroidism.

The rate of urinary creatine excretion, varying widely in normal individuals, is a less conclusive measure in the diagnosis of hyperthyroidism than of hypothyroidism, in which creatinuria is greatly diminished or absent.

The creatine tolerance test reliably reveals the muscular disturbances associated with Graves' disease, but is of little help in diagnosing hypothyroidism in children (1).

THYROXIN TEST

The studies of Wilkins and his co-workers (4) on the high sensitivity of hypothyroid children to thyroid medication form the basis of this test. Changes in the urinary creatine excretion or in the serum cholesterol level are the criteria of the subject's response to the test, which consists of a daily, uniform dose of ¼ to ½ grain of desiccated thyroid, by mouth, or of a single dose of thyroxin, parenterally. The effect of this test dose upon the serum cholesterol is the best means of differentiating between the hypothyroid and the normal child (4).

PROCEDURE

The most practicable form of the thyroxin test is as follows: At least two determinations of the serum cholesterol (page 137) are performed during a control period of 3 to 5 days, after which a single injection of 2 or 5 mg. of thyroxin is given intramuscularly.

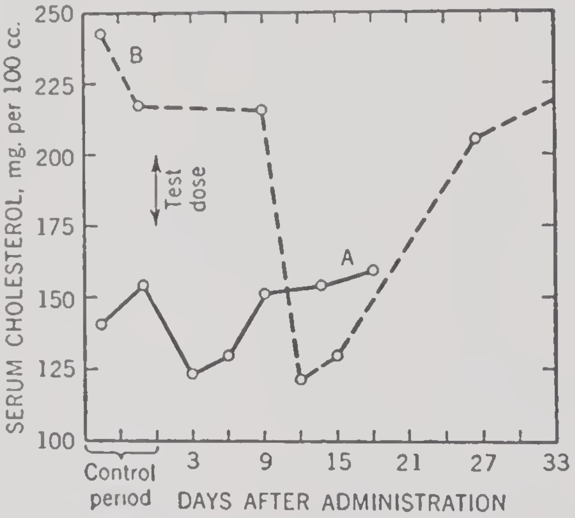

Fig. 42. Response to single-dose thyroxin test, as judged by changes in the level of serum cholesterol. A: Normal, 8 year old boy, test dose of 5 mg. thyroxin. B: 1 year old male cretin, test dose of 2 mg. thyroxin. Drawn from figures given by Wilkins, Fleischmann, and Block (4).

Following the day of injection, the serum cholesterol level is determined every third or fourth day until the cholesterol has returned to the original level (a period of 40 to 60 days). Ordinarily, a 2 week follow-up of the cholesterol level suffices to differentiate between the normal and hypothyroid response.

In normal children, the fall in serum cholesterol is small and transient, the decrease varying from 16 to 47 mg. per hundred cubic centimeters, with an average of 31 mg. The minimum level is usually reached between the third and sixth days following the injection (Fig. 42).

In hypothyroidism, the drop in the cholesterol level is marked and sustained, varying from 121 to 185 mg. per hundred cubic centimeters, with an average decrease of 157 mg. It usually becomes apparent between the third and seventh day (Fig. 42). There is no constant or direct relationship between the degree of sensitivity to thyroxin, as measured by the fall in the cholesterol level in serum, and the original cholesterol concentration, as determined during the control period.

PARATHYROID GLANDS

Diagnosis of decreased or increased parathyroid function by means of chemical tests rests primarily on the demonstration of changed electrolyte levels in the blood and urine (Table 68). Test methods and their evaluation are discussed in Chapter VII. Hypoparathyroidism may also be suggested by evidence of increased galvanic excitability of the peripheral nerves (page 388).

TABLE 68
Biochemical Data Suggestive of Abnormal Parathyroid Activity

	Characteristic changes (above or below normal) in		
Constituent measured	Hypoparathyroidism	Hyperparathyroidism	Test method outlined on page
Total calcium in serum......	Decreased	Increased	182
Ionized calcium in serum....	Decreased	—	192
Inorganic phosphorus in serum	Increased	Decreased	202
Serum alkaline phosphatase..	—	Increased*	211
Urinary calcium............	Decreased	Increased	197
Urinary phosphorus.........	Decreased	Increased	—

* In cases with bone involvement.

PITUITARY GLAND

The physiologic functions by which the pituitary earned its epithet "master gland of the endocrine system" are functions of the anterior lobe. It is known that the thyroid and parathyroid glands, the adrenal cortex, the gonads, and the pancreas function under the regulatory influence of the anterior pituitary hormones. The rate of thyroid, adrenocortical, and gonadal activity is most intimately connected with the central control station of the pituitary. Thus, identical metabolic disorders may develop either as a result of pathology located in one of the controlled glands or in the anterior pituitary exercising the control. The various active hormones or principles of the anterior lobe, listed according to their known physiologic effects, are as follows: (*1*) growth hormone; (*2*) thyrotropic hormone; (*3*) adrenotropic hormone; (*4*) glycotropic, or insulin-antagonizing hormone; (*5*) ketogenic factor; (*6*) parathyrotropic factor; (*7*) pancreatropic factor, (*8*) gonadotropic hormones.

TABLE 69

Biochemical Data Suggestive of Abnormal Anterior Pituitary Activity

Constituent or reaction measured	Characteristic changes (above or below normal) in		Discussed on page
	Hypopituitarism	Hyperpituitarism	
Basal metabolic rate	Decreased	Increased	65
Specific dynamic action of protein	Decreased	Increased	—
Fasting blood sugar	Subnormal	Above normal	99
Glucose tolerance	Increased	Decreased	103
Insulin sensitivity	Increased	Decreased	120
Epinephrine hyperglycemia	Decreased	—	125
Water–electrolyte balance	—	Disturbed	228
Urinary 17-ketosteroids	Decreased	Increased	464
Urinary gonadotropic (pituitary-like) substances	Decreased	Increased	452

Evidence of deficient anterior pituitary function usually consists of a decrease of those endocrine activities which depend on the normal pituitary "adenotropic" stimulus. The production of one, of several, or of all factors may become deficient. The potential biochemical changes associated with anterior lobe deficiency are listed in Table 69.

The opposite results are obtained in patients with an overactive anterior pituitary lobe, usually the result of pituitary tumors. In the majority of such patients, clinical and biochemical manifestations indicate that various anterior pituitary hormones are involved, even if the increased production was originally confined to only one of the hormonal factors, for instance, the corticotropic hormone released by an isolated tumor of the basophilic cells. Because of the proclivity of tumors to invade or press upon neighboring structures, any function of either lobe may become deranged when the lesion expands, leading to functional disorders in all peripheral endocrines governed by the pituitary-hypothalamic mechanism (5).

The main clinical manifestations of anterior lobe deficiency are dwarfism, pituitary cachexia or Simmond's disease, and acromicria, the antithesis of acromegaly.

The well-known syndromes which result from overactivity of the anterior pituitary lobe are gigantism, acromegaly, and Cushing's syndrome, or pituitary basophilism.

The evidence of the indirect test methods listed in Table 69 does not tell whether the observed metabolic disorder (for example, an abnormal increase in the urinary 17-ketosteroids) is due to primary organic disease of the pituitary, with only secondary involvement of the peripheral endocrine function (for example, of the adrenal cortex), or is due solely to some pathology in one of the peripheral endocrines itself. The ideal data for a complete study of a given endocrine disorder would include quantitative determination of blood and urine levels of the various pituitary hormones.

Unfortunately, the available technics only permit urinary assay of the gonadotropic principles of the pituitary gland. According to Heller and Nelson (6), these principles are: (*1*) the gametogenic principle, commonly called the follicle-stimulating hormone (F.S.H.) because its main function is to cause follicular growth in the female and spermatogenesis in the male; and (*2*) the interstitial cell stimulating hormone, commonly called the luteinizing hormone (L.H.), which elicits estrogen secretion in females and androgen production in males. The available methods of determination are biologic assays measuring the responses provoked in immature rats or mice by urinary extracts containing the gonadotropic material (7a). Follicle-stimulating and luteinizing principles may be conveniently estimated together as gonadotropins (7b).

It has been shown that nonchorionic substances of gonadotropic character are present in the urine of adults (8). In children of both sexes they do not appear in appreciable amounts before the eleventh or twelfth year (9), although small amounts have been found at a considerably earlier age (10).

The greatest clinical value of such assays is in the differentiation between primary and secondary gonadal dysfunction (page 472).

The posterior lobe of the pituitary exerts a regulatory influence on vascular tension, muscle tone, and diuresis through the secretion of two active principles. Oxytocin (pitocin, obstetric pituitrin) causes contraction of the gravid uterus. Vasopressin (pitressin) raises arterial tension, stimulates contraction of unstriped muscles, and has a strong antidiuretic action.

Deficient secretion of the posterior lobe, or a hypothalamic lesion, results in diabetes insipidus.

Impaired function of both lobes of the pituitary, or a hypothalamic lesion, leads to dystrophia adiposogenitalis (Fröhlich's syndrome).

ADRENAL GLANDS

Adrenal Medulla

Epinephrine, or adrenalin, the active substance elaborated by the adrenal medulla, was the first hormone to be obtained in crystalline form, and is now prepared synthetically. The chief physiologic activities of the medullary hormone are: (*1*) sympathicomimetic action, which is similar to stimulation of the sympathetic nervous system, causing, for example, elevation of blood pressure, acceleration of pulse rate, and increase of cardiac output; (*2*) metabolic action, such as elevation of basal metabolic rate, mobilization of muscular glycogen, hyperglycemia, and glycosuria.

One simple means of obtaining information on the activity of the adrenal medulla is to test the response of the blood pressure, pulse rate, or blood sugar to the subcutaneous or intramuscular injection of 0.5 cc. of epinephrine. As compared to the normal reaction, the rise in blood pressure, pulse rate, and blood sugar is abnormally high and prolonged in subjects in whom the activity of the adrenosympathetic system is unusually high. In contrast, a weak response to a dose of epinephrine indicates that in the individual tested the parasympathetic tone is dominant. The interpretation of the effect of epinephrine on the blood sugar is conditioned by the fact that the response also depends on the amount and character of the glycogen stores in the liver and muscles (page 125).

Indications for these tests in children are rather limited. Results of the tests may help to demonstrate an imbalance in the vegetative regulations, due to an increase or decrease of adrenosympathetic activity. A clear-cut differentiation of such vegetative imbalances cannot always be made. They may be a constitutional variant, or the result of an actual endocrine disorder. Medullar hyperadrenalism occurs in children as a result of tumors arising from the medulla (neuroblastomas). Table 70 lists the tests which are useful in the evaluation of adrenomedullar activity.

TABLE 70
Evaluation of Functional Activity of Adrenal Medulla

Constituent or reaction measured	Characteristic changes (above or below normal) in		Test method outlined on page
	Hypoactivity	Hyperactivity	
Basal metabolic rate	Decreased	Increased	65
Fasting blood sugar	Low	High	99
Glucose tolerance	Increased	Decreased	103
Glycosuria	None	Present	—
Blood pressure response to epinephrine	Decreased	Very strong	—
Epinephrine hyperglycemia	Absent or weak	Marked	125

Adrenal Cortex

Unlike the adrenal medulla, which is not essential for life, the products elaborated by the cortex are indispensable for the maintenance of health and life. The various functions of the cortex are carried on by a number of hormones, which have been shown to be compounds of steroid nature. A report on the isolation of the adrenosteroids from the adrenal extracts, on their partial synthesis, and on the study of their intermediary metabolism may be found in one of the recent reviews of the subject, for example, that by Pfiffner (11).

The rationale of the procedures for testing cortical function becomes clear when the chemical properties and physiologic effects of the cortical hormones are understood. A recent paper by Talbot and Sobel (12) presents this information in succinct form. These authors, adopting Albright's views, divide adrenocortical hormones into three groups, in accordance with their physiologic action. These are:

(*1*) Water–electrolyte hormone. The action of this hormone is almost identical with that of the synthetic steroid, 11-desoxycorticosterone. It is representative of a type of cortical hormone which chiefly affects the metabolism of water, sodium, potassium and chloride, the state of body hydration, the electrolyte content of extracellular and intracellular body water, and the efficiency of the blood circulation.

(*2*) Protein–carbohydrate hormone. This hormone, exemplified by Kendall's compound E (11-dehydro-17-hydro-oxycorticosterone), promotes protein catabolism and the formation of sugar from the protein catabolites (gluconeogenesis from protein). Because of this relation to sugar metabolism, this hormone is commonly termed the "S hormone."

(*3*) Androgenic hormones. These hormones, with somewhat the same characteristics as testosterone, promote masculinization and nitrogen anabolism. The latter property has caused them to be labeled "N hormone." Their effect on protein metabolism is essentially the opposite of that produced by the S hormone. Chemically, the N hormone is related to adrenosterone, a steroid isolated from the adrenal cortex.

The manifestations of cortical deficiency or hyperactivity consist of a combination of symptoms, each deriving from an altered production of any one of the three cortical principles. Diagnosis of such endocrine disorders is relatively easy when the patient exhibits the fully developed clinical features of hypoadrenocorticism or hyperadrenocorticism. In subclinical and mild forms of deficiency or overactivity, however, diagnosis largely depends on the functional evaluation of each type of cortical activity, by means of laboratory tests.

Hypoadrenocorticism. Its classic form is known as Addison's disease. The deficiency involves all the cortical hormones, and possibly lack of medullar secretion as well. Lack of the water–electrolyte hormone is evidenced by the profound changes in the electrolyte and water balances, mainly as a result of impaired renal function (13,14). According to Loeb (13), there is an unusually high urinary excretion of sodium and chloride. Due to this abnormal loss, the concentration of sodium in the serum may fall from a normal value of about 140 mEq. per liter to 100 mEq., and the serum level of potassium may rise to about 10 mEq. per liter, somewhat more than double the normal concentration. A decrease in serum chloride or bicarbonate, or in both, accompanies the changes in cation content of the serum. The blood level of urea usually parallels that of potassium. However, according to Loeb, "there is often no increase in serum potassium in patients with Addison's disease."

Dehydration due to excess loss of water can be demonstrated by the reduction in plasma volume and by the increase in plasma protein and nonprotein nitrogen. All these abnormalities in water and electrolyte distribution can be restored almost to normal by eliminating their essential cause, namely, the pathologic loss of sodium and chloride, and, with them, of water. Administration of corticosterone or desoxycorticosterone, or ingestion of sodium chloride brings the desired retention of sodium and water (Table 71). This was the first function test employed in the clinical diagnosis of cortical deficiency.

TABLE 71

Blood Changes Correlated with Salt Depletion and Salt Feeding in a Patient with Addison's Disease

Date, 1932	Serum				N.P.N., mg./100 cc.	Blood pressure	Remarks
	Na	K	Cl	HCO_3			
	mEq./L.						
July 19	123.3	5.3	88.6	21.8	39.0	80/55	Shortly after admission
July 26	107.8	7.1	72.7	21.5	45.0	65/48	Critically ill; no therapy up to this time
Aug. 2	133.0	5.1	93.8	27.5	20.6	84/60	Single dose of eschatin, then NaCl daily
Nov. 14	139.9	4.6	107.3	24.3	20.0	112/74	7 Gm. NaCl daily, and diet; up and about at home

From Loeb (13).

Another function test, devised by Cutler, Power, and Wilder (15), uses the opposite criterion, namely, the decrease in urinary excretion of sodium chloride following sodium and chloride deprivation under controlled conditions. This procedure, apart from the serious risks involved, calls for prolonged metabolic study, and the authors no longer consider it "to be entirely satisfactory." Instead, they recommend the test procedure devised by Robinson, Power, and Kepler (page 460). Since the method is comparatively simple and eliminates the need for salt deprivation, as well as of the time-consuming metabolic study, it is described below. The test seems feasible for children.

Direct tests, such as assays in blood or urine of the cortical salt and water hormone, are not available for clinical use.

Lack of S hormone (protein–carbohydrate hormone) can be recognized by tests which demonstrate that gluconeogenesis is abnormally decreased, and that the glycogen stores in the liver are depleted. According to Lewis *et al.* (16), the fasting blood sugar is low, there is a tendency to hypoglycemia following dietary restriction of the carbohydrate intake and during infections, and the glucose tolerance is increased, giving flat tolerance curves. In addition, the effect of epinephrine on the blood sugar is greatly diminished, whereas the sensitivity to insulin is so great that serious hypoglycemic reactions are not uncommon. A low basal metabolic rate is also found as a result of S hormone deficiency. The colorimetric assay of urinary substances resembling 11-oxycorticosteroids, as described by Talbot *et al.* (17), is a direct method for estimating S hormone production. Since only urinary corticosteroids with a 17-hydroxyl group are measured by this procedure, the authors believe that the results reflect specifically on the rate of production of the S hormone. Clinical application of the test is still in the preliminary stage.

Deficient adrenocortical production of N hormone and of androgenic hormone is revealed by an abnormally low output of urinary 17-ketosteroids, the excretory transformation products of this group of hormones. Standards referred to age and sex must serve as the basis for differentiation between normal and abnormal excretion rates. A description of the test will be found on page 464.

Patients with Addison's disease suffer from a deficiency of the various adrenocortical hormones; in other clinical forms of chronic hypoadrenocorticism, however, only one type of hormone is lacking. Cases have been reported (Table 73, page 459) which combined a deficiency of the water and electrolyte hormone and an excess formation of androgens.

Acute hypoadrenocorticism is caused by adrenal hemorrhage (Waterhouse-Friderichsen syndrome). It may develop as the result of trauma at birth, in the course of septicemia (particularly meningococcemia), or in the toxemia of severe burns.

The known causes of chronic hypoadrenocorticism are structural damage to the cortex due to malformation, atrophy, or tuberculosis, and functional impairment in the wake of infectious diseases (hypoadrenia). Table 72 shows the relation between cortical deficiency and the biochemical changes.

Hyperadrenocorticism. Manifestations of adrenocortical hyperfunction share two common features: involvement of the genital sphere due to excessive discharge of androgens, and a "bewildering complex" of clinical signs and symptoms caused by an imbalance between the activities of the other cortical hormones (18a). Conclusions as to etiology, and proposals for a functional classification of hypercortical disease have been rather disputed, partly because the clinical signs of hormonal changes vary not only according to the type and amount of hormone secreted but also with the age and sex of the patient. However, there is some reasonable assurance of certain correlations between the clinical picture and the state of hormone production (12,19,20a), and these form the basis for a grouping of the various syndromes as follows:

TABLE 72
Biochemical Data on Adrenocortical Insufficiency

Constituent or reaction measured	Changes (above or below normal)	Cortical hormone essentially involved*	Test method outlined on page
Na in plasma	Low	W–E	225
Cl in plasma	Low	W–E	461
K in plasma	Elevated	W–E	223
HCO_3 in plasma	Low	W–E	240
Urea in blood	Elevated	W–E	373
Protein in serum	Increased	W–E	159
N.P.N. in serum	Increased	W–E	161
Plasma volume	Diminished	W–E	233
Na in urine	Increased	W–E	223
Cl in urine	Increased	W–E	463
Urea in urine	Low	W–E	371
17-ketosteroids in urine	Reduced	N+A	464
Basal metabolic rate	Decreased	S	65
Fasting blood sugar	Low	S	99
Glucose tolerance	Increased	S	103
Insulin tolerance	Decreased	S	120
Diuresis following water test	Impaired	W–E	460
Interrelation between diuresis, and excretion of urea and Cl	W–E	Deranged	461

* W–E: Water–electrolyte hormone. S: S hormone. N+A: N hormone + androgenic hormone.

(*1*) Clinical manifestations due to excess formation of N hormone (protein anabolic or androgenetic hormone). In this group are included: (*a*) premature sexual and physical development, e.g., pseudohermaphroditism, precocious physical development; (*b*) increased or decreased virility, e.g., precocious sexual development, appearance of adult masculinity in both male and female children, or of masculinity in the adult female and femininity in the adult male. All of the above are referred to as the adrenogenital syndrome.

TABLE 73

Metabolic Changes Associated with Various Forms of Hyperadrenocorticism

Clinical syndrome*	Biochemical changes	Test method outlined on page
Cushing's syndrome, if not due to cancer (12)	Decreased glucose tolerance	103
	Increased insulin resistance	120
	Normal or moderately increased urinary excretion of 17-ketosteroids	464
	Increased urinary excretion of 11-oxycorticosteroids	457
Virilism (12)	Extremely high urinary excretion of 17-ketosteroids	464
	Normal or slightly increased urinary excretion of 11-oxycorticosteroids	457
	Increase in ratio of urinary 17-ketosteroids to urinary 11-oxycorticosteroids	—
Pseudohermaphroditism in females (18c)	Excess urinary excretion of androgenic steroids	464
Adrenal pubertas praecox and macrogenitosomia (combining features of hyperfunction and hypofunction of adrenal cortex) (23–25)	Decreased level of plasma Na	225
	Decreased level of plasma Cl	461
	Increased level of plasma K	223
	Increased level of plasma N.P.N.	161
	Low blood sugar level	99
	Excess urinary excretion of androgenic steroids	464

* Numbers in parentheses are reference numbers.

(2) Clinical manifestations due to excess formation of S hormone (antimetabolic or gluconeogenetic hormone). These include metabolic disturbances, such as obesity, hypertension, hyperglycemia, osteoporosis, and Cushing's syndrome.

(3) Clinical manifestations due to excess formation of the cortical N and S hormones. These comprise Cushing's syndrome associated with virilism or other sexual changes.

(*4*) Clinical manifestations due to excess formation of N hormone, and lack of the water–electrolyte hormone. In this group are included premature physical development, precocious sexual development, obesity with changes in the water–salt balance as observed in Addison's disease, adrenal pubertas praecox, and macrogenitosomia.

Biochemical data on some typical hyperadrenocortical syndromes will be found in Table 73. The anatomic changes in the adrenals in any of the syndromes may range from simple hyperplasia to carcinoma of the cortex (18b). Judging from the fractional analysis of urinary 17-ketosteroids, there seems to be a significant difference in the partition of these substances in hyperplasia and in malignancy (21,22). It is well to remember that a number of adrenogenital syndromes, as well as Cushing's, can be produced by extra-adrenal pathology, as, for example, pathology of the pituitary, the ovaries, or the pineal body. Typical clinical pictures of Cushing's syndrome have also been observed when no pathology could be verified on postmortem examination.

TEST FOR ADDISON'S DISEASE

The method, devised by Robinson, Power, and Kepler (26), is based on observations that most patients with Addison's disease do not experience normal diuresis and excrete excessive amounts of sodium chloride, but retain urea (page 456). In providing objective evidence of these abnormalities, the test could be considered as a measure of renal efficiency. Actually, it gages that particular cortical function which governs renal ability to maintain the metabolic balance of water and electrolytes. The test consists of two parts: one determines the rate of diuresis; the other measures and correlates electrolyte and urea changes in the blood and urine. Obviously, the test results cannot lead to any conclusion except if renal disease has been ruled out.

Water Test. On the day preceding the test, the patient should (*1*) eat ordinary meals but omit extra salt; (*2*) neither eat nor drink anything after 6 P.M., although up to this time water may be drunk as desired; (*3*) empty his bladder at 10:30 P.M. (this urine being discarded). Thereafter, all urine voided until 7:30 A.M. of the following day (test day) is collected, the volume measured, and the specimen saved for chemical analysis, if this is necessary later.

On that day (test day) breakfast is omitted. The patient should void again at 8:30 A.M., and immediately thereafter be given 20 cc. of water per kilogram of body weight; this he is asked to drink within 45 minutes. Beginning at 9:30, the patient should empty his bladder every hour until 12:30 P.M., remaining in bed until after the fourth specimen is obtained. Each specimen is kept separately, and the volume of the largest specimen is measured when collection is complete. No food or drink is given until the 4 specimens are collected and blood has been drawn for the electrolyte test, should this be necessary.

Interpretation. If the volume of any single specimen voided during the morning is greater than the volume of urine voided during the night, the test result is negative, indicating the absence of Addison's disease.

If the volume of the largest specimen voided during the morning is less than the volume of urine voided during the night, the response to the test is positive, and Addison's disease may or may not be present. The electrolyte test must then be done to establish the diagnosis.

Electrolyte and Urea Test. Blood is obtained while the patient is still fasting, and the plasma is analyzed for urea (page 373) and for chlorides (page 461). The night urine specimen is analyzed for urea (page 371) and for chlorides (page 463). The figures obtained in these 4 determinations and in the water test are used to calculate the result by the equation:

$$A = \frac{\text{urinary urea (mg. \%)}}{\text{plasma urea (mg. \%)}} \times \frac{\text{plasma Cl (mg. \%)}}{\text{urinary Cl (mg. \%)}} \times \frac{\text{vol. (cc.) day urine}}{\text{vol. (cc.) night urine}}$$

where *day urine* is the largest specimen voided during day, and *night urine* is the entire amount voided from 10:30 P.M. to 7:30 A.M. of the test day.

Interpretation. If the value of A in the equation is greater than 30, the patient probably does not have Addison's disease.

If the value is less than 25, the patient probably has Addison's disease, provided the presence of nephritis can be ruled out.

Results suggesting cortical insufficiency may also be obtained in patients with Simmond's disease, in which there is severe hypofunction of the pituitary (20b).

Determination of Chlorides in Serum. The procedure devised by Kramer (27) is a modification of the methods of Van Slyke (28) and of Wilson and Ball (29). Its advantages are that deproteinization is unnecessary and that only 0.1 to 0.2 cc. of serum is needed for each analysis.

Reagents.

(*1*) Silver nitrate solution. Dissolve 16.989 Gm. silver nitrate in water and make up to 100 cc.

(*2*) Saturated aqueous solution of ferric alum. Shake the crystals with water at room temperature, then add enough nitric acid to change the color from red to yellow.

(*3*) Chloride reagent. Transfer into a 100 cc. volumetric flask 3 cc. normal silver nitrate solution, 40 cc. saturated aqueous ferric alum solution, and 55 cc. concentrated nitric acid; when the solution has come to room temperature, add conc. HNO_3 up to the mark.

(*4*) 0.01 *N* (approximately) thiocyanate solution. Dissolve 0.811 Gm. sodium thiocyanate, or 0.972 Gm. ammonium thiocyanate in distilled water to make 1 liter. The solution should be standardized against the chloride reagent each time it is used.

Technic. To 1 cc. of the chloride reagent in a small test tube is added 0.2 cc. of serum, and the contents are thoroughly mixed. The tube is then placed on a hot plate at a temperature of 90 to 100 C. until the solution clears up and assumes a light yellow color; this usually requires 1 to 2 hours. After cooling, the excess silver nitrate is determined by titrating with 0.01 *N* thiocyanate. The end point is the appearance of a reddish brown color which persists for at least 1 minute. The thiocyanate solution should be standardized against the same amount of chloride reagent as is used for the actual determination.

Calculation.

$$\frac{\text{Mg. Cl}}{\text{per 100 cc. serum}} = 0.3546 \times \frac{3}{A} \times \frac{(A - \text{cc. } 0.01\ N \text{ thiocyanate}) \times 100}{\text{cc. sample taken}}$$

$$\frac{\text{Mg. NaCl}}{\text{per 100 cc. serum}} = 0.5846 \times \frac{3}{A} \times \frac{(A - \text{cc. } 0.01\ N \text{ thiocyanate} \times 100)}{\text{cc. sample taken}}$$

where A is cubic centimeters of 0.01 *N* thiocyanate required for 1 cc. of chloride reagent, and cc. 0.01 *N* thiocyanate is the actual volume used in titrating the excess silver nitrate (about 0.03 cc. less than that delivered from the buret).

$$\text{mEq. Cl per L.} = \frac{\text{mg. Cl per 100 cc.}}{3.5457}$$

$$\text{mEq. NaCl per L.} = \frac{\text{mg. NaCl per 100 cc.}}{5.8454}$$

Normal chlorine values in adults and children are 350 to 380 mg. per 100 cc. of serum, or 100 to 107 mEq. per liter of serum.

Normal sodium chloride values are 570 to 620 mg. per 100 cc. of serum, or 75 to 85 mEq. per liter of serum.

Simplified Determination of Chlorides in Urine. The method described is that of Folin (30).

Reagents.

(*1*) Standard silver nitrate solution. Dissolve 7.28 Gm. silver nitrate in water and dilute to a volume of 250 cc. 1 cc. is equivalent to 10 mg. sodium chloride.

(*2*) Indicator. Dissolve 100 Gm. ferric ammonium sulfate in 100 cc. water and add 200 cc. concentrated nitric acid.

(*3*) Standard ammonium thiocyanate solution. Dissolve 4 Gm. ammonium thiocyanate in 200 cc. water. Standardize this solution as follows: Transfer 10 cc. of the standard silver nitrate solution to a beaker, add 20 cc. water and 5 cc. of the indicator, and titrate with the thiocyanate solution from a buret until the characteristic reddish end point is reached. On the basis of the figure obtained, prepare 200 cc. of an ammonium thiocyanate solution that is equivalent to the standard silver nitrate solution.

Technic. 5 cc. of urine are transferred to a beaker with a pipet, and 20 cc. water, 5 cc. indicator, and 10 cc. standard silver nitrate solution are added. While stirring with a glass rod, the surplus silver nitrate is titrated with the standard thiocyanate solution until the first faint but unmistakable brown or reddish color is obtained. Since the color disappears on standing or continued stirring, the very first appearance of the color must be noted.

Calculation.

$$\text{Mg. NaCl per 100 cc. urine} = (10 - \text{cc. thiocyanate used}) \times 200$$

DETERMINATION OF NEUTRAL 17-KETOSTEROIDS IN URINE

It is believed that the urinary 17-ketosteroids are excretory transformation products of the androgenic hormone of the male gonads (testosterone), and of the androgens of the adrenal cortex. Thus, assay for urinary 17-ketosteroids is not an exact test for androgens as such, although in many cases it may parallel the biologic test for the latter (31). Sex and age of the subject influence the urinary output of 17-ketosteroids: at about the age of 8 years, the male gonads enter as a source of ketosteroid excretion, whereas in females the output remains an index of the activity of the adrenal cortex alone.

In view of the fact that the contribution of the testes to the 17-ketosteroid excretion is only small, the correlation of the rate of urinary 17-ketosteroid excretion with adrenocortical function seems justified. According to Albright (32), if one considers only those steroids which are normally produced in the body and eliminated as 17-ketosteroids, the excretion of these substances in the urine, by and large, is an index rather than a measurement of the production of androgens by the adrenal cortex, and these, in turn, are a rough index of N hormone production.

PROCEDURE

The method described is the one devised by Talbot and coworkers (21, 33–35). It is based on the color reaction introduced by Zimmermann (36)—the red color produced by *m*-dinitrobenzene with substances containing the CH_2CO grouping, in alkaline alcoholic solution. The 17-ketosteroids only are measured, not those ketosteroids having the ketone group in another position. Estrone is not included in the assay, for while it is a 17-ketosteroid, it is removed during the extraction process.

Reagents.

(*1*) Stannous chloride.

(*2*) Carbon tetrachloride.

(*3*) 10 per cent sodium hydrosulfite solution. Dissolve 10 Gm. sodium hydrosulfite ($Na_2S_2O_4$) in 100 cc. of normal sodium hydroxide.

(*4*) 2 *N* sodium hydroxide. Dissolve 80 Gm. sodium hydroxide in water and make up to 1 liter.

(*5*) 10 per cent sodium hydroxide.

(*6*) 0.5 *N* hydrochloric acid.

(*7*) Glacial acetic acid.

(*8*) Absolute and 95 per cent ethyl alcohol.

(*9*) Girard's reagent T (trimethylacethydrazide ammonium chloride, Eastman).

(*10*) *m*-Dinitrobenzene reagent. For purposes of purification, dissolve 20 Gm. metadinitrobenzene (m.p., 89–89.5 C.) in 750 cc. of 95 per cent ethyl alcohol. Heat the solution gently to 40 C., add 100 cc. of 2 *N* sodium hydroxide, and after 5 minutes cool the mixture and dilute with 2,500 cc. distilled water. Collect the precipitate that has formed, in a Buchner funnel, wash thoroughly with water, and dry with suction. Recrystallize from 120 and 80 cc. of absolute alcohol. A 1 per cent alcoholic solution of the purified substance mixed with an equal volume of 2 *N* sodium hydroxide should remain colorless after standing 1 hour. The reagent consists of a 1.16 per cent solution of the purified *m*-dinitrobenzene in absolute alcohol, or 11.6 mg. per cubic centimeter of alcohol. It remains stable for 10 to 14 days when stored in a brown bottle in the refrigerator.

(*11*) Ethyl ether. Should be free of impurities, such as peroxides.

(*12*) 2.5 *N* alcoholic potassium hydroxide. Dissolve 9 Gm. potassium hydroxide, with the aid of stirring, in 50 cc. absolute ethyl alcohol. Filter the solution, with suction, through a Whatman filter no. 50. Check the concentration by titration against 2.5 *N* acid, using methyl orange as indicator. If necessary, adjust with alcohol. The solution keeps for 2 to 5 days if stored in the refrigerator.

Apparatus.

(*1*) Continuous extractor. The authors recommend the apparatus devised by Hershberg and Wolfe (37).

(*2*) Photoelectric colorimeter.

Technic. A 24 hour urine specimen is collected, using 7 cc. of concentrated hydrochloric acid as a preservative. The volume of the specimen is measured, and concentrated hydrochloric acid is added in the proportion of 150 cc. of acid to 1 liter of urine; 0.7 Gm. stannous chloride is added to prevent emulsion. The cold mixture is then placed in the continuous extraction apparatus and is simultaneously heated, hydrolyzed, and extracted with carbon tetrachloride for 7 hours. The solvent is then distilled off (it may be used repeatedly) and the residue is dissolved in 100 cc. ethyl ether. The etheric solution is transferred to a separatory funnel and washed 2 times with 25 cc. lots of 10 per cent sodium hydrosulfite solution, 2 times with 25 cc. lots of normal sodium hydroxide, 2 times with 25 cc. lots of 0.5 N hydrochloric acid, and 3 times with 25 cc. lots of distilled water.

After the ether is evaporated on a steam bath, the dry residue is dissolved in absolute alcohol, 1 cc. of alcohol for each 100 cc. of urine extracted. This is referred to as the *crude extract.*

The colorimetric assay of the 17-ketosteroids is performed on this crude extract. However, precautions must be taken against possible errors caused by the presence of other chromogens which contribute to the intensity of the color reaction when the *m*-dinitrobenzene reagent is introduced. The likelihood of error may be greatly reduced by one of two procedures. (*1*) Prior to the colorimetric assay, the crude extract is treated with Girard's reagent, and the nonketonic fraction, containing the bulk of the interfering chromogens, is thereby removed. What remains is the ketone fraction containing the 17-ketosteroids. (*2*) The colorimetric assay is carried out on the untreated crude extract, and the results are calculated by using the color correction equation. Depending on which procedure is preferred, the treatment of the crude extract is carried through as follows.

Girard's Procedure. An aliquot portion of the neutral crude extract is transferred to a 250 cc. Erlenmeyer flask. The ether is then evaporated, and the residue is thoroughly dried on a steam bath. To the dry residue 0.5 cc. of glacial acetic acid and 200 cc. of Girard's reagent are added. The flask is stoppered with tin foil, and the mixture is heated for 10 minutes on a boiling water bath. The mixture is allowed to cool, 40 cc. of ice water and 3 cc. of 10 per cent sodium hydroxide are added, and the mixture is extracted 4 times with 40 cc. lots of ethyl ether. The combined ether extracts are then washed 3 times with 20 cc. lots of distilled water. These water washings are combined with the original aqueous phase which remains after the ether extraction, 1 cc. concentrated sulfuric acid and 20 cc. ethyl ether are added, and the mixture is allowed to stand for at least 2 hours. Then 1 cc. concentrated sulfuric acid is added just prior to the beginning of the extraction, which is carried out 4 times with 40 cc. lots of ethyl ether. The ether extracts are again combined and evaporated to dryness on a steam bath. The residue is dissolved in absolute ethyl alcohol to the original volume of the aliquot portion of the crude extract used. This *final alcoholic extract*, representing the ketonic fraction of the crude extract, should be assayed promptly, since it changes on standing.

Colorimetric Assay. Approximately 0.2 cc. of the alcoholic extract (ketone fraction) obtained by Girard's procedure is transferred into a colorimeter tube or cell. If less than 0.2 cc. is used, the volume is brought to 0.2 cc. with absolute alcohol. Then 0.2 cc. of *m*-dinitrobenzene reagent and 0.2 cc. of 2.5 N alcoholic potassium hydroxide are added. Simultaneously, 2 blanks are prepared, each containing 0.2 cc. absolute alcohol, 0.2 cc. *m*-dinitrobenzene reagent, and 0.2 cc. of 2.5 N alcoholic potassium hydroxide solution. The contents of each tube are thoroughly mixed, and the tubes are placed in a water bath at 25 C. in a darkened room or closet for 80 minutes. The tubes are then removed from the bath, and 20 cc. of 95 per cent alcohol are added to each tube. The solutions are read within 3 to 20 minutes in the electrocolorimeter, with green filter no. 520.

To one of the developed and diluted blanks is also added the same amount of the final alcoholic solution as was used in the test sample. The reading of this blank (G_2) must be used to correct the reading of the sample (G_1) for the color of the alcoholic extract solution.

Calculation. The 17-ketosteroid content of the sample solution is computed from a calibration curve prepared by colorimetric measurement of standard solutions of crystalline androsterone, containing from 0.05 to 0.3 mg. per sample. The milligrams of sterone which correspond to the blank reading G_2 in the calibration curve are subtracted from the value obtained from the reading G_1 in the curve. The difference represents the corrected milligrams of 17-ketosteroids in the sample solution, expressed in milligrams of androsterone.*

Since construction of the calibration curve provides also the value of K, the constant of proportionality, the result may be calculated by the equation:

$$C_c = K(\log G_2 - \log G_1)$$

---

* 100 μg. of androsterone represent the International Androgenic Unit; its biologic equivalent is the International Capon Unit (I.C.U.).

where C_c is the corrected concentration of steroids in the sample solution; K has been determined as 0.067, under the conditions of the procedure outlined above, and using the Evelyn electrocolorimeter. For the technic of calibration, see page 187.

The final result is calculated as milligrams of 17-ketosteroids (expressed as androsterone) contained in the 24 hour sample of urine.

Color Correction Equation (35,31). The colorimetric assay is carried out on 0.2 cc. of the crude urine extract. The technic is the same as described for the colorimetric determination of the 17-ketosteroids in 0.2 cc. of the ketone fraction obtained by Girard's procedure. Two readings are taken, one with green filter no. 520, another with blue filter no. 420. To eliminate the contribution of interfering chromogens to the color intensity, correction is made by the equation:

$$C = \frac{K_i\,(G - B)}{K_i - K_s}$$

where C is corrected reading; G is extinction coefficient* (E_G) obtained for the urine extract–*m*-dinitrobenzene solution with the green filter; B is extinction coefficient (E_B) obtained for the urine extract–*m*-dinitrobenzene solution with the blue filter; K_i is the ratio E_G/E_B for a sample of the interfering chromogens; and K_s is the ratio E_G/E_B for samples of crystalline 17-ketosteroid solutions.

The value of K_i is found by determining the ratio E_G/E_B of the nonketone fraction obtained by the Girard separation. This value, according to Talbot *et al.* (35), "represents the color characteristics of a 'pure' solution of the interfering chromogens of urine extracts." K_s is estimated with pure solutions of crystalline dehydroisoandrosterone.

The values of K, as established by Talbot *et al.*, are 0.6 for K_i, and 2.2 for K_s. It should be possible to make use of these figures, the authors believe, provided that the reagents and the colorimetric filters are similar to the ones used by them. In principle, however, it is advisable for each laboratory to determine its own K values.

According to Soffer (20c), the results of the colorimetric assay of the crude urine extract may be considered as sufficiently accurate if the reading obtained with the green filter is less than 1.5 times the value obtained with the blue filter. In such a case, neither Girard's procedure nor the color correction equation need be used.

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* The extinction coefficient is the log of the blank galvanometer reading minus the log of the test galvanometer reading.

INTERPRETATION

The urinary output of neutral 17-ketosteroids normally varies with the age and sex of the subject. According to Talbot *et al.* (38) and Nathanson *et al.* (9), who studied the excretion in boys and girls of different ages, the output is less than 1 mg. per day in children up to the age of 8 years. Between the ages of 8 and 18, the average output rises from less than 1 mg. per day to about 9 mg. Adult women excrete an average of 6.8 mg. per day, adult males 11 mg. The average output per day of normal children, adolescents, and adults is given in Table 74 and Figure 43.

TABLE 74

Average Normal Output of Neutral Urinary 17-Ketosteroids at Various Ages

Age, years	Output, mg./day	Age, years	Output, mg./day
3	0.15	12	3.4
4	0.3	13	4.3
5	0.4	14	5.3
6	0.5	15	6.3
7	0.65	16	7.2
8	0.95	17	8.1
9	1.4	Adult women	6.8
10	1.9	Adult men	11.0
11	2.6		

From Talbot *et al.* (38).

An abnormally high excretion of 17-ketosteroids can be easily recognized by comparison with the normal figures for the age and sex. The conditions that are usually associated with, and that are often to be detected by, an excessive output of 17-ketosteroids have already been mentioned (page 458). A few examples of actual measurements may be of interest. Thus, in Cushing's syndrome, when caused by adrenal cancer, the output may be as high as 74 mg. per day. When the syndrome is not the result of cancer, it is often associated with a "high normal" output of ketosteroids—from 10.4 to 19.7 mg. per day (31). An excretion rate of 12.6 to 176 mg.

per day has been observed in patients with the adrenogenital syndrome, both in males and females (31). Talbot *et al.* (39) found that normal children between the ages of 5 and 8½ years who are exceptionally tall and of accelerated physical development, but "with no data suggesting diseases of the gonads, adrenal cortex, or central nervous system," often have an output between 0.8 and 7.8 mg. per day, which is higher than that of children of normal size and development and corresponding chronologic age.

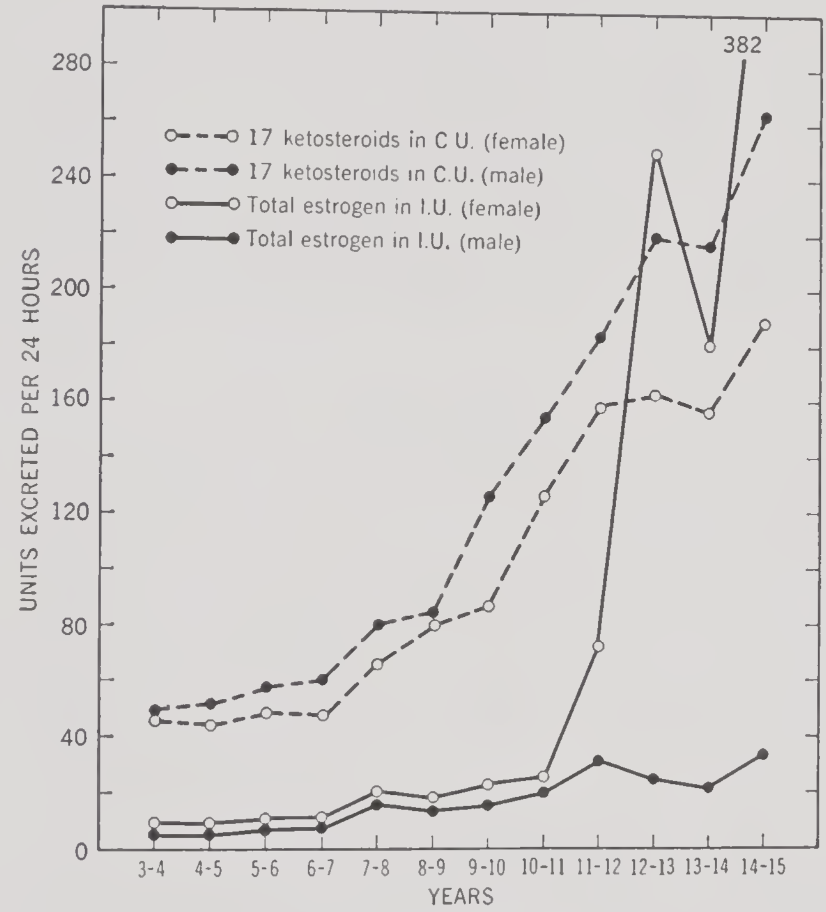

Fig. 43. Normal urinary excretion of 17-ketosteroids and estrogen by children. C.U. = color unit, one color unit being equivalent by colorimetric titration to sixty micrograms of crystalline androsterone. I.U. = international unit, one international unit of estrogen activity being equivalent in biologic activity to 0.1 microgram of crystalline estrone. From Nathanson, Tower, and Aub (9).

Up to the age of 11 it is impossible to detect abnormally low excretion, since the output, under normal conditions, is low from birth to about the age of 10. Talbot *et al.* (38) state: "If they have passed their twelfth birthday, children should be excreting at least 1 mg. a day." Addison's disease is the most important clinical condition in which 17-ketosteroids assay reveals an abnormally low rate of excretion: zero in females, and 4 to 5 mg. in males. A complete absence of androsterones in the urine has also been found in both male and female patients with panhypopituitarism (Simmond's disease) (31). In acromegaly, the values obtained run from 2.9 to 10.6 mg. in females, and approximately 8.3 mg. in males. In this condition, therefore, there is apparently a slight reduction in the excretion rate, whereas the rate may be very low in hypothyroidism (31).

In dwarfed and sexually retarded children with anterior pituitary insufficiency, the excretion of 17-ketosteroids is below normal, particularly after the age of 12 years. Values of 0.2 to 1.3 mg. per day, found in patients with an average age of 12¼ years (38), correspond to figures normally found in children 4 to 6 years old.

TESTES AND OVARIES

The functions of the testes and ovaries are not limited to that of spermatogenesis and follicular growth, respectively. The gonads have endocrine functions as well, the interstitial cells of the testes (Leydig's cells) secreting androgens and the interstitial or thecal cells of the ovaries secreting estrogen.

The gonadal endocrine functions develop under and are sustained by the stimulating influence of the anterior lobe of the pituitary, transmitted by the interstitial cell stimulating hormone (I.C.S.H.) which is commonly called the luteinizing hormone (L.H.). The internal secretion of the gonads may therefore be disturbed as a result of altered functional capacity of the gonads themselves, or, as is more frequently the case, because of a disordered pituitary stimulating mechanism.

The androgens secreted by the testes belong to the same group of steroids as the adrenocortical androgens; their degradation products include the 17-ketosteroids, and are excreted in the urine. As has already been stated (page 464), a method has been developed for assaying the urinary 17-ketosteroids, whose origin is adrenocortical as well as gonadal.

Estrogen is also one of the steroid compounds, and undergoes a chemical transformation similar to that of the androgens before it is excreted in the urine. Biologic methods employing animals as test objects, determine the estrogenic activity of the urinary steroids derived from estrogen. It has been possible to ascertain the course of urinary estrogen excretion from birth to puberty and maturity by means of such biologic assays.

According to Nathanson *et al.* (9), both boys and girls between the ages of 3 and 7 excrete a small but constant amount of 17-ketosteroids and estrogen, and sex differences are small. With the approach of puberty (8–11 years), there is a steady increase in the excretion of these gonadal hormones, as may be seen from Figure 43 and Table 74 (page 469). The 17-ketosteroid excretion rises in both boys and girls, but more rapidly in boys, whereas the increase in estrogen excretion becomes pronounced in girls only. By about the age of 12, when the secondary sex characteristics begin to appear, the 17-ketosteroid excretion prevails in boys, the estrogen excretion in girls. Approximately 1½ years before the menarche, this estrogen excretion becomes cyclic (9), but there are no obvious cycles of 17-ketosteroid excretion in females. Normal adolescent girls and nonpregnant women rarely excrete more than 1,000 I.U. of estrogen per day (39).

In making use of the results of urinary sex hormone determinations for the clinical diagnosis of hypergonadism and hypogonadism, it should be remembered that neither data on sex hormone excretion nor clinical manifestations are necessarily evidence of the functional capacity of the gonads themselves. The condition found may be the result of primary gonadal dysfunction, i.e., of pathologic processes in the testes or ovaries; but it may also be the result of secondary gonadal dysfunction, due to primary disturbances of those functions of the pituitary which govern gonadal activity. Obviously, this applies only to postpuberal conditions, since these functions of the pituitary are not fully established until puberty.

The urinary assay of the pituitary gonadotropic hormones, particularly of the luteinizing hormone, would provide the means of differentiating between primary and secondary gonadal dysfunction. But while such methods are available (page 452), they hardly lend themselves for use as quick diagnostic tests.

Primary deficiency of the gonads (hypogonadism) is not only evidenced by low urinary values of 17-ketosteroids or estrogens, but also by a greatly increased output of pituitary sex hormones. Conversely, secondary gonadal dysfunction, i.e., dysfunction caused by lack of pituitary activity, is characterized by a normal amount of gonadal hormone but reduced values of pituitary gonadotropic hormones.

When excessive amounts of androgens or estrogens appear in the urine, the disturbances may have originated in the gonads or adrenal cortex (primary hypergonadism); but it is also possible that the primary disturbance occurred in the basophilic cells of the pituitary, which then stimulated the said endocrines to produce abnormally high amounts of hormones (secondary hypergonadism). Only in the latter case will the urine contain an unusually high concentration of pituitary sex hormones. It is impossible to diagnose primary or secondary dysfunction of the gonads on the basis of clinical symptoms alone.

Wilkins' summary (40) of the results obtained with such tests in children of abnormal sexual development illustrates the practical application of assays of urinary excretion of sex hormones as follows:

Sexual precocity may be precipitated by influences from the brain or the pituitary which provoke gonadal activity at an unusually early developmental stage and lead to precocious sexual maturation. The urinary findings in such cases are: (*1*) excretion of 17-ketosteroids or estrogens in amounts normally found in adolescents and adults; and (*2*) the presence in the urine of follicle stimulating and/or luteinizing hormone in quantities normally excreted by adolescents.

If the premature sexual development is due to primary gonadal or adrenocortical pathology, the characteristic findings are: (*1*) excretion of 17-ketosteroids or estrogens in amounts exceeding those normally found in the urine of adults; and (*2*) absence of appreciable amounts of follicle-stimulating and luteinizing pituitary hormone, such as normally occurs at the approach of puberty.

Adrenal pathology may cause sexual precocity by releasing abundant material of androgenic or estrogenic activity. Depending on the character of the lesion, various abnormalities may occur in the excretion of the sex hormones. In the adrenogenital syndrome (page 459), in which excessive androgenic effects prevail, 17-ketosteroid excretion will be the outstanding finding, whereas in

Cushing's syndrome the urine will contain only slightly higher than normal amounts of 17-ketosteroids or estrogen. Finally, when feminizing tumors of the adrenal cortex are the underlying pathology, as for example in gynecomastia, excessive amounts of estrogen will be found in the urine.

Retarded sexual development and its various causes are often difficult to recognize and to differentiate clinically. As Wilkins (40) has pointed out, the separation of merely delayed adolescence from a true, permanent sexual infantilism is aided by repeated determinations of the urinary androgens. If a steady increase in the elimination of 17-ketosteroids or estrogen occurs over appropriate periods of time in a child who is failing to show normal sexual maturation, the condition may be considered as a temporary one, and not the result of organic pathology in the gonads or pituitary.

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CHAPTER XVI

Miscellaneous Tests

DETERMINATION OF BLOOD CLOTTING TIME

Rodda Test (1). The child's heel, sponged with ether, is punctured with an automatic lancet, the blade being set at about 0.5 cm. so as to produce a free flow of blood without the slightest pressure. The first drop of blood is discarded. The second drop is received onto a clean, dry watch glass, about 1.5 inches in diameter, and containing a no. 6 lead shot. A second watch glass is inverted over the first, and the glasses are gently tilted every 30 seconds until the shot no longer rolls about but is fixed in the clot.

Normal average clotting time, as determined by this method, is 7 minutes, the range being 5 to 10 minutes. A clotting time of more than 10 minutes represents delayed coagulation. These values apply to infants, children, and adults.

In the newborn there is a physiologic prolongation of the clotting time from the third to the seventh day of life, as a result of prothrombin deficiency (page 52).

While the Rodda test is a simple one, and particularly convenient for testing infants and young children, Howell (2) states: "It is now generally recognized that all methods in which the specimen of blood is obtained by pricking or stabbing the skin are unreliable on account of the possibility of admixture of more or less thromboplastic substance from the tissues."

More accurate results are obtained when the sample of blood is removed by venipuncture, as in the test that follows. Nevertheless, except when results nearest to the "absolute" clotting time are desired, as in the diagnosis of hemophilia, in which methods using venous blood are to be preferred, the Rodda test gives satisfactory results for clinical work.

Howell's Test (2). The method described is the modification of Lee and White (3). Blood is withdrawn by venipuncture, using a sterilized syringe that has been thoroughly rinsed with normal saline solution. Every effort should be made to enter the vein promptly at the first insertion of the needle, to use a minimum of suction, and to withdraw the blood and the needle as rapidly as possible. The needle is removed and approximately 1 cc. of blood is placed in each of 2 clean, saline-rinsed glass tubes (Widal tubes), about 8 mm. in diameter. One tube is rotated endwise every 30 seconds, and that point at which the blood no longer flows from its position but maintains its surface contour when inverted, is taken as the end point. The lapse of time between the end of the venipuncture and this end point is the clotting time.

As determined by this method, the normal clotting time in children and adults ranges between 6 and 15 minutes.

DETERMINATION OF BLEEDING TIME

The method described is that of Duke (4). A fairly deep skin puncture wound is made in the ear lobe. Several drops of blood should flow out spontaneously. The blood is blotted up with filter paper at intervals of 30 seconds, care being taken not to touch the skin. With each blotting the size of the blot decreases, until bleeding finally ceases. The lapse of time between the moment the incision was made and the cessation of bleeding is the bleeding time.

As determined by this method, normal bleeding time ranges between 1 and 7 minutes.

TEST FOR STABILITY OF THE PLATELETS

The method described is that of Quick (5). Blood is withdrawn by venipuncture and rendered incoagulable by the addition of 0.5 cc. of 0.1 *M* solution of sodium oxalate for each 3 cc. of blood. The oxalated plasma is divided into 2 equal portions, and transferred into centrifuge tubes. One tube is centrifuged at 1,000 r.p.m., the other at 3,000 r.p.m. The two samples are then recalcified by adding 0.2 cc. of 0.0125 *M* solution of calcium chloride.

If the blood is normal, both samples will clot at about the same time after recalcification. Hemophilic blood, however, is significantly affected by the speed of centrifugation; the portion centrifuged at

3,000 r.p.m. has a considerably shorter clotting time than the portion centrifuged at 1,000 r.p.m.

DIFFERENTIAL DIAGNOSIS OF DEFECTS IN CLOTTING MECHANISM

In the laboratory diagnosis of hemorrhagic diseases, the first step is to differentiate between conditions which are due to: (*1*) Quantitative changes in the platelets. Such changes are shown by platelet counts. (*2*) Increased permeability of the capillary wall.

TABLE 75

Hemorrhagic States Due to Defects in the Blood Clotting Mechanism and How to Identify the Defect

Defect	Pathogenesis	Associated diseases	Test results	Test method outlined on page
Thromboplastin deficiency	Lack of thromboplastic substance in platelets; abnormal resistance of platetets to fragmentation	Hemophilia	Positive centrifugation test Normal bleeding time	478 478
Prothrombin deficiency	Defective vitamin K supply	Hemorrhagic disease of newborn	Lengthened prothrombin time	49
	Faulty vitamin K absorption	Obstructive jaundice; severe intestinal disorders	Lengthened prothrombin time	49
	Defective vitamin K synthesis in liver	Severe liver damage (hepatitis, obstructive jaundice, infection, cirrhosis, neoplasma); salicylate intoxication	Lengthened prothrombin time	49
Fibrinogen deficiency	Congenital	Hereditary fibrinopenia	Decreased serum fibrinogen	161
	Acquired	Severe liver diseases; nutritional deficiency diseases; chloroform poisoning; extreme destruction of bone marrow	Decreased serum fibrinogen	161
Increase in antithrombin (heparin)	—	Anaphylactic shock	—	—

Based on Quick's classification (6).

This is revealed by capillary resistance tests (page 310), or, in scurvy, by the determination of the blood ascorbic acid level (page 276). (*3*) Disturbances in the clotting mechanism. These are revealed by lengthened clotting time (page 477).

If it has been established that defective coagulation is the probable cause of the bleeding tendency, the factor or phase of the clotting process which is at fault must next be found. The current concept of the factors involved in the mechanism of blood coagulation has been summarized on page 49. The data presented here (Table 75) combine a classification of hemorrhagic states due to coagulation defects and a list of test methods which will demonstrate the specific character of the defect in each group.

RED BLOOD CELL SEDIMENTATION TEST

The methods commonly used in adults require amounts of blood which can be obtained only by venipuncture. Since venipuncture is undesirable in infants and small children, micromethods have been developed which use capillary blood from the finger tip.

For normal and moderately increased sedimentation values, the results of both macromethods and micromethods are in good agreement. This does not hold true for high sedimentation rates (Fig. 44). According to Landau (7), however, "when there is a question as to normal or increased sedimentation and as to the course of the curve, the results obtained with the micromethods are amply sufficient." The two micromethods described are recommended for use in children. The first one, devised by Smith (8), bars the use of capillary sendimentation tubes, the second one, by Landau (7), makes use of them.

PROCEDURES

Smith's Method (8). *Apparatus.* All the equipment is commercially available.

(*1*) Blood pipet, similar to the Sahli hemoglobin pipet, graduated at 0.04 cc. and 0.1 cc.

(*2*) Small test tube.

(*3*) Long capillary pipet (elongated medicine dropper) with rubber bulb attached; the capillary portion should be long enough to reach the bottom of the sedimentation tube.

(*4*) Sedimentation tube, similar to the Cutler tube (9). It has a diameter of 2 to 5 mm. and is graduated in 50 mm. divisions, the capacity in the measured area being less than 0.25 cc.

Technic. The anticoagulant to be used is decided upon at the outset of the test. In contrast to citrate, heparin does not materially affect the distribution of water and electrolytes in cells and plasma. Heparin, therefore, is the anticoagulant to be preferred, particularly when both sedimentation rate and red cell volume are to be determined with one and the same blood sample and in the same tube (page 484).

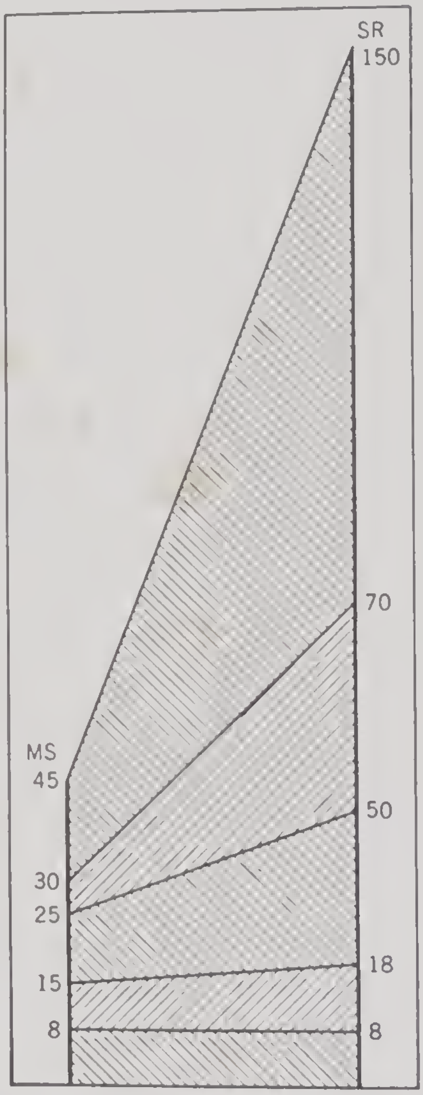

Fig. 44. Sedimentation rate. Microvalues (MS), in minutes, obtained with Landau's method and the corresponding macrovalues (SR) obtained with Westergren's method. From Landau (7).

A drop of a solution of 50 mg. heparin in 2 cc. distilled water is transferred into a small test tube and dried.

If sodium citrate is used, 0.04 cc. of a 5 per cent solution of sodium citrate is transferred into a small test tube by means of the blood pipet. The pipet is moistened by drawing up the citrate solution repeatedly before transferring the 0.04 cc. into the test tube.

Blood is then drawn from the punctured finger tip by 3 consecutive aspirations of 0.1 cc. each—0.3 cc. of blood in all—and transferred to the test tube, and the tube is shaken so that blood and anticoagulant are thoroughly mixed. The heparinized or citrated blood is drawn from the test tube with the capillary pipet and transferred to the sedimentation tube. The latter is filled gradually by releasing the blood by gentle pressure on the bulb as the pipet is slowly withdrawn from the bottom of the tube until the uppermost zero mark is reached. Any excess above this mark is easily removed with the same pipet. The sedimentation tube is then allowed to stand at room temperature. A reading of the red cell level is taken after 30 minutes and after 1 hour. The sedimentation rate is measured by the depth, in millimeters, of clear plasma which has formed at the top of the vertical column of blood cells.

Normal values, in children between the ages of 12 days and 14 years, as obtained with this method, are 1 to 8 mm. (average, 4.2 mm.) after 30 minutes, and 3 to 13 mm. (average, 9.1 mm. after 1 hour. Any value over 15 mm. is abnormal.

Landau's Method (7). *Apparatus.* All the equipment is commercially available.

(*1*) Capillary pipet, 12.5 cm. long and with a bore of 1 mm. diameter, expanded into a bulb or mixing chamber 3 cm. from the upper end. The pipet has marks 12.5 mm. and 62.5 mm. from the lower tip, with the intervening space graduated in millimeters.

(*2*) Aspirator. It consists of a metal screw with a milled head and its collar; the screw is 3.5 cm. long and fits into a 4.5 cm. long piece of thick rubber tubing. When screwed down, the entire length of the aspirator is 5.5 cm.

(*3*) Metal rack with a rubber-cushioned floor and adjustable rubber-cushioned cap. A hidden spring device in the floor permits the base to be depressed and holds the pipet sealed.

Technic. The aspirator is screwed down and its rubber end is fitted on the capillary pipet. Holding the pipet in the palm of the hand, daggerlike, so that the thumb and index finger control the screw, a 5 per cent solution of sodium citrate is drawn up into the pipet to the 12 mm. mark by revolving the screw. Thereupon, blood is drawn from the pricked finger or toe to the 62.5 mm. mark, taking care to avoid air bubbles. The blood column is then drawn into the mixing chamber of the pipet until the lower meniscus is a few millimeters below the chamber. The blood is alternately depressed and raised 7 times, thus emptying and filling the chamber, to ensure thorough mixing; this should not take more than 30 seconds. Finally, the blood column is screwed down until the upper meniscus reaches the mark just below the mixing chamber. The aspirator is disengaged, the lower tip of the pipet is pressed into the rubber floor of the rack, and the upper end of the pipet is placed beneath the cap of the rack. After 1 hour, the millimeter level to which the red cells have fallen is read.

Normal values after 1 hour, as obtained by this method, are 1 to 6 mm. in children under 2 years of age, and 1 to 9 mm. in children over 2 years of age. Any value over 15 mm. is abnormal.

INTERPRETATION

In order to determine the extent to which oligocythemic anemia may be responsible for an increased sedimentation rate, the red blood cell count or the relative cell volume of the blood being tested should be determined at the same time as the sedimentation test. An inverse ratio exists between the rate at which the cells settle and their number. One of the procedures devised to correct for the factor of anemia, e.g., the method suggested by Wintrobe and Landsberg (10), may be used, but it is simpler and safe enough to judge for oneself to what extent the red cell count or the cell volume percentage, if it is abnormal, may have influenced the result of the sedimentation test.

Increased sedimentation rates have been observed in such conditions as acute and chronic inflammations, malignant tumors, jaundice, and severe liver disease. The nonspecificity of the test should always be remembered. The following pathologic states in children are typically associated with an abnormally increased sedimentation rate; septicemia, active tuberculous processes, anemias, leukemias, active rheumatic fever, and malignant growths.

A reduced sedimentation rate is rare in children, and has little diagnostic significance. The few conditions in which it has been observed include sickle-cell anemia and extreme dehydration.

DETERMINATION OF RELATIVE RED BLOOD CELL VOLUME (PACKED RED CELLS)

In addition to the commonly used hematocrit measurements, the precedure recommended by Smith (8) merits the particular attention of pediatricians. It is particularly useful when carried out in combination with the microsedimentation test according to the author's directions.

The anticoagulant preferably used is heparin, although the test may also be performed on citrated blood.

After the sedimentation test, as devised by Smith, is finished, the sedimentation tube with the heparinized or citrated, settled blood is transferred into the centrifuge. The tube is then centrifuged at 2,500 r.p.m. for 30 minutes. The millimeters of packed cells multiplied by 2 constitutes the cell volume percentage.

Normal values in children, as determined by this method, are over 37.5 per cent (average, 43.5) with heparinized blood, and over 30 per cent (average, 36.0) with citrated blood.

RED CELL FRAGILITY TEST

The degree of resistance of red cells to a decrease in the salt content of their environment serves as a criterion of their fragility. Erythrocytes suspended in slightly hypotonic solutions will take in water and swell, without any diffusion of hemoglobin. But when the strength of the surrounding salt solution is decreased still further, the red cells reach their "hemolytic volume" (11) and hemoglobin diffuses out. Since not all red cells of any individual's blood have the same hemolytic volume, the solution's osmotic pressure must be lowered beyond the point at which the most fragile cells begin to hemolyze, in order to bring about the hemolysis of all the suspended corpuscles.

By recording the salt concentrations which cause minimum, maximum, and intermediate degrees of hemolysis, respectively, the span of osmotic resistance, or the spread of fragility, may be determined. The results can be charted as osmotic resistance curves.

The fragility of the erythrocytes may increase or decrease under pathologic conditions. This is evidenced by (*a*) the occurrence of all degrees of hemolysis at salt concentrations which are below or above the normal values, or (*b*) a prolongation of the span of osmotic resistance beyond one or the other of its normal extremes.

As a clinical method, the test was first used by Ribierre (12). For ordinary purposes, it is still so performed. This method requires venous blood, employs sodium chloride for preparing the serial hypotonic dilutions, and determines the degree of hemolysis by visual observation. More accurate results are obtained with buffered solutions of sodium chloride (13a) and electrocolorimetry (13b). For studying large groups of patients, a screening test (14) has been devised which uses capillary blood from the finger and reduces the number of hypotonic test solutions from 12 to 3.

PROCEDURE

The method described is a modification of the original technic of Ribierre (12), as proposed by Giffin and Sanford (15).

In a Wassermann rack with 2 rows of holes, 12 holes in a row, are placed 24 small tubes. The front row of 12 tubes is used for the blood to be tested, the back row for a control with normal blood. Both sets of 12 tubes are numbered 25 to 14. With a capillary pipet or Mohr pipet as many drops of a 0.5 per cent solution of sodium chloride are pipetted into each tube as is indicated by its number. Water is added to each tube with a similar pipet to bring the number of total drops up to 25 in each tube. The percentage strength of the sodium chloride solutions in the various tubes is calculated by multiplying the number on the tube by 0.02.

One drop of whole venous blood is transferred into each tube. The blood may be dropped directly from the syringe with which it was withdrawn. It may also be taken from a suspension prepared as follows: 2 to 3 cc. of blood taken by venipuncture are placed in a 15 cc. graduated centrifuge tube containing 5 cc. of 2 per cent sodium citrate in physiologic saline solution. The tube is corked, inverted, placed in the centrifuge, and revolved until the cells have been packed to the tip. The supernatant is removed, and the packed cells are diluted with physiologic saline solution up to the 2 or 3 cc. mark, depending upon the amount of blood taken originally. The control set is prepared in similar fashion, using blood of normal fragility.

After the tubes have stood for 1 hour at room temperature, the results of the test are ascertained as follows:

(*1*) The tubes in both rows are examined macroscopically for hemolysis. The numbers of the samples which show commencing, well-marked, and complete hemolysis, respectively, are recorded, the intensity of red discoloration of the supernatant fluid serving as criterion.

(*2*) The sediment of the samples with most extensive hemolysis is examined microscopically for remaining residue of nonhemolyzed cells.

(*3*) The results obtained with the patient's blood and the control blood are compared.

The screening test, devised by Smith (14), offers a number of simplifications. Only 3 dilutions of hypotonic solution of sodium chloride are needed, namely 0.375, 0.35, and 0.325 per cent sodium chloride. To each of the 3 tubes 2 or 3 drops of blood from the pricked finger tip are added. The tubes are then corked, inverted, and allowed to stand in a refrigerator until sedimentation has occurred. A control with normal blood is set up in a similar way. The degree of hemolysis and the residue of unhemolyzed cells are determined as described above. If any clotting occurs, the tube must be centrifuged at low speed for a few minutes before the degree of hemolysis can be estimated by comparison with the corresponding control.

INTERPRETATION

In specimens of normal blood, hemolysis usually begins in 0.48 to 0.42 per cent saline solutions (minimal resistance), is well marked at concentrations of about 0.39 per cent, and complete at approximately 0.3 per cent (maximal resistance). The normal span between minimal and maximal resistance amounts to differences of about 0.15 per cent. Results obtained in children and adults are practically identical.

Increased fragility of erythrocytes is indicated when hemolysis sets in at higher concentrations than are required normally and is complete in dilutions which cause only slight liberation of hemoglobin from normal cells. The span of resistance is usually shortened. Such lowered resistance to the action of hypotonic solutions occurs in congenital hemolytic jaundice (16). Hemolysis may begin in the tubes containing 0.58 per cent sodium chloride, and may be complete at 0.45 per cent, the osmotic strength at which the minimal resistance of normal red cells is usually found. Occasionally, increased fragility is also present in patients with acute hemolytic anemia associated with infection or ingestion of drugs (14).

Decreased fragility is characterized by (*a*) Hemolysis starts in weaker salt solutions than those causing minimum hemolysis in normal red cells. (*b*) The span of resistance is prolonged, beginning at normal concentrations or at abnormally dilute solutions. Although hemolysis often begins in the same salt dilution as for the control blood (e.g., at 0.48 per cent), the increased hemolysis which normally occurs with advancing dilution does not take place. While the control might show marked and complete hemolysis in 0.42 and 0.3 per cent solutions, respectively, abnormally resistant cells might fail to be markedly hemolyzed down to the 0.26 per cent solution, and become completely hemolyzed only in 0.2 per cent sodium chloride. Furthermore, increased resistance is also indicated by a residue of unhemolyzed cells frequently found in those tubes which, macroscopically, show complete hemolysis. With such residue miss-

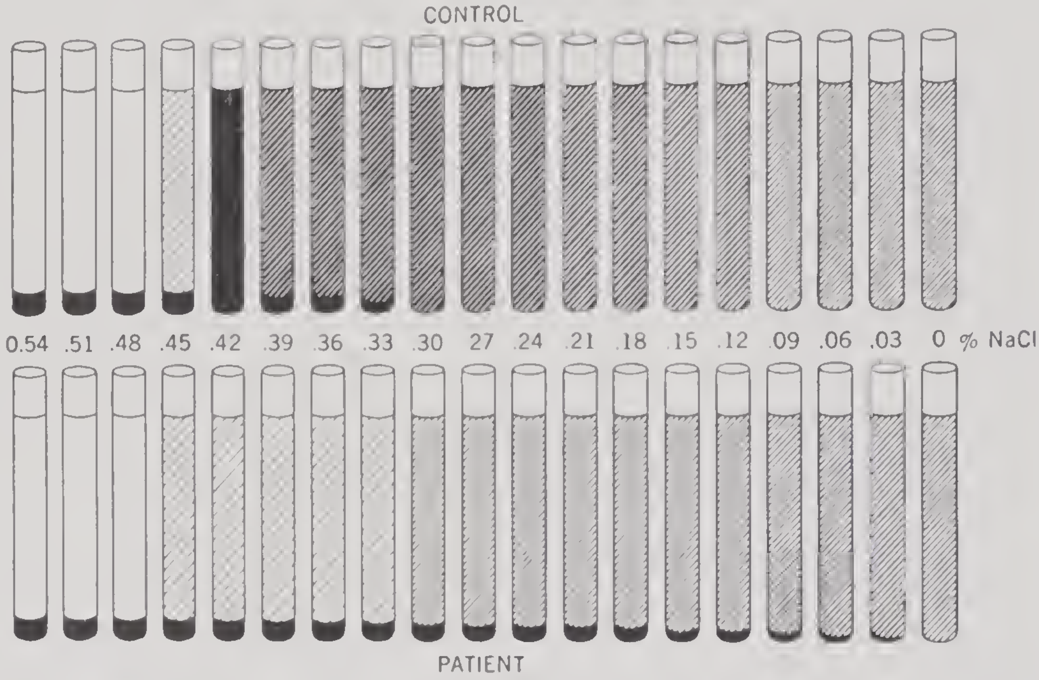
CONTROL
0.54 .51 .48 .45 .42 .39 .36 .33 .30 27 .24 .21 .18 .15 .12 .09 .06 .03 0 % NaCl
PATIENT

Fig. 45. Results of red cell fragility test with blood from a patient with Mediterranean anemia and with normal control blood. Clear tubes, no hemolysis; light hatching, slight hemolysis; heavy hatching, marked hemolysis. Note the slow development of hemolysis and persistence of corpuscular residue in the patient's blood. From Wintrobe *et al.* (17).

ing in the controls below the concentration of 0.3 per cent, they may persist in much more dilute concentrations, sometimes down to 0.03 per cent, if the cells are abnormally resistant (17).

Decreased fragility is an important diagnostic finding in sickle cell anemia (18), in Mediterranean anemia (19,20), and in iron deficiency anemia (14). Smith (20) emphasizes that the fragility test is particularly useful as a single method for detecting the mild type of Mediterranean anemia. He also proposes the screening test as a simplified method for bringing to light entire families with this disease (14).

In secondary anemias, the results of the fragility test are irregular.

PREPARATION OF STANDARDS FOR MEASURING TURBIDITY

The method described is that of King and Haslewood (21). These standards, preferably permanent, are needed for the evaluation of the thymol turbidity test and for the turbidimetric estimation of protein in body fluids, particularly in urine (22). In the latter procedure, 2.5 cc. of urine are diluted to 10 cc. with 3 per cent sulfosalicylic acid and the resulting turbidity is compared visually with that of the standards.

The standards consist of a suspension of formazin in gelatin; they are standardized at values of albumin ranging from 10 to 100 mg. per hundred cubic centimeters in 10 standard tubes, and are made up as follows:

(*1*) Gelatin. 65 Gm. pure gelatin are dissolved at about 90 C. in 500 cc. water. The white of 1 egg in approximately twice its volume of water is vigorously stirred into the solution, and the mixture is heated, with constant stirring, on a boiling water bath for 1 hour. It is then filtered through a large, coarse paper in a heated funnel. The clear, slightly yellow filtrate is kept liquid at about 50 C. for the preparation of the standards.

(*2*) Formazin. 25 cc. of an aqueous solution (10 Gm. per 100 cc.) of methenamine (hexamethylenetetramine) are added to 25 cc. of a solution (1 Gm. per 100 cc. water) of hydrazine sulfate. The mixture is shaken in a stoppered bottle, and then left at room temperature for at least 15 hours. The precipitate of formazin which results is carefully mixed by gentle shaking until it is evenly dispersed throughout the liquid.

(*3*) Formazin–gelatin suspension. 14.5 cc. of the formazin are added to 100 cc. of the gelatin solution, together with 0.3 cc. of 40 per cent formaldehyde to ensure permanent "setting." This gelatin suspension of formazin is equivalent to a concentration of albumin (precipitated with sulfosalicylic acid) of 100 mg. per hundred cubic centimeters.

(*4*) Standards. The gelatin–formazin suspension is diluted with clarified gelatin (containing 0.3 cc. of 40 per cent formaldehyde per 100 cc.) to give standards corresponding to other albumin concentrations. The following mixtures of gelatin and gelatin–formazin suspension (Table 76) are made in small tubes of 75 × 100 mm. bore.

TABLE 76
Mixtures of Gelatin and Formazin–Gelatin Suspensions

Tube No.	Gelatin solution, cc.	Gelatin-formazin suspension, cc.	Turbidity in mg. albumin per 100 cc
1	3.6	0.4	10
2	3.2	0.8	20
3	2.8	1.2	30
4	2.4	1.6	40
5	2.0	2.0	50
6	1.6	2.4	60
7	1.2	2.8	70
8	0.8	3.2	80
9	0.4	3.6	90
10	0	4.0	100

When cold, the tubes are stoppered with corks cut level with the top of the tube. The stoppered ends are dipped in melted paraffin wax and allowed to cool.

The standards may be checked against albumin solutions standardized by nitrogen determination. They should be mounted in a wooden rack painted black. Comparison is best made by looking at the tubes against a strip of white cardboard, with a transverse black line, that has been fastened to the rack. If the turbidity of the sample exceeds the 100 mg. standard, the sample under investigation must be diluted with a measured volume of water in the case of urinalysis, or with a measured volume of buffer in the case of the thymol turbidity procedure (page 47).

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